QUALITATIVE AND QUANTITATIVE ANALYSIS OF THE CANNABINOID CONTENT OF COMBUSTED CANNABIS PLANT TISSUE

A Thesis Submitted to the College of Graduate Studies and Research in Partial Fulfillment for the Requirements for the degree Master's of Science in the College of Pharmacy and Nutrition

University of Saskatchewan

Saskatoon

By

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ABSTRACT

Marihuana continues to be a controversial topic in today's society. The plant material is used recreationally as a stupefacient and has a purported medicinal use. In fact, anecdotal information about its medicinal properties has been such that Health Canada has recently started an initiative to provide a well characterized supply of plant tissue to researchers interested in examining its potential medicinal properties. Simultaneously this same material is being provided for those subjects who are licensed to use marihuana as a medicine.

In order to further study marihuana and its component cannabinoids it is essential to be able to discern both the cannabinoid content in the product being supplied and more importantly the cannabinoid profile in the delivery system. At present the most common route of administration is via inhalation of the combusted plant material. Consequently methods capable of measuring the cannabinoid content in combusted plant material would be very useful in order to make meaningful study of the pharmacokinetics of the cannabinoids delivered by this route.

Investigations were carried out to develop a method and study the volatile constituents of combusted marihuana plant material in a semi-enclosed environment. Thus the hypothesis of this research is that qualitative and quantitative information can be obtained from the combustion products of cannabis plant tissue. The method relies upon the solid phase extraction of smoke arising from the combustion of plant material in a variety of combustion chambers. The combustion chambers were designed to reflect the current marihuana "paraphernalia" in use as well as a high efficiency in vitro system. Both the qualitative and quantitative levels of a limited number of cannabinoids

were evaluated before and after combustion. A quantitative Liquid Chromatography Ultra Violet (LC-UV) detector method was validated for the analysis of a selected group of cannabinoids (Δ9-tetrahydrocannabinol (THC), cannabinol (CBN), cannabidiol (CBD) and tetrahydrocannabinolic acid (THCA) . When this method was applied to plant material available through the medical marijuana initiative the following observations were made on the combustion products:

MATERIAL ANALYZED	CBD μg/mL	CBN (μg/mL)	THC (μg/mL)	THCA (μg/mL)
PLANT	BLQ	BLQ	BLQ	890
COMBUSTED	20	14	420	28

- 5 mg plant tissue extracted using a validated HPLC-UV method
- 5 mg plant tissue combusted in a closed combustion chamber.
- BLQ: below level of quantification (<12.5 μg/mL)

These results sparked further research into the quantitative transformation of cannabinoids during the combustion process. In this regard, it was shown that THC, CBD and CBN all could be recovered at approximately a 90% ratio upon combustion However, THCA was thermally converted such that very little remains after combustion and furthermore, its degradation product, THC can only account for 50% of the THCA. The latter observation is important since there has been a prevailing thought that THCA is quantitatively transformed during combustion to THC while in fact this transformation is approximately 50%. This finding must be considered when pharmacokinetic studies are carried out using inhalation of combusted plant material as the delivery system.

A further finding of this research is that not all popular combustion devices yield identical quantitative cannabinoid profiles. In this regard, the simpler “pipe” systems

result in the highest yields of heat transformed cannabinoids while the vaporizer systems have the lowest cannabinoid yields.

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LIST OF ABBREVIATIONS

AUC ..... area under curve

AVG ..... average

BLQ ..... below limit of quantification

CBC ..... cannabichromene

CBD ..... cannabidiol

CBG ..... cannabigerol

CBN ..... cannabinol

CME ..... cannabis medicinal extract

GC ..... gas chromatography

HPLC ..... high performance liquid chromatography

LC ..... liquid chromatography

LLQ ..... lower limit of quantification

MEOH ..... methanol

MS ..... mass spectrometer

N/A ..... not applicable

RCMP ..... Royal Canadian Mounted Police

RO ..... reverse osmosis

STDEV ..... standard deviation

SPE ..... solid phase extraction

THC ..... Δ^9 tetrahydrocannabinol

THCA ..... tetrahydrocannabinolic acid

UV ..... ultra-violet

%CV.....coefficient of variation

1 INTRODUCTION

1.1 Background

The subject of marihuana is a controversial topic in today's society. It is legalized in some parts of the world and illegal in others. Research on marihuana reached its peak during the 1970's and 1980's but then it was largely abandoned for a number of years. However, the plant from which marihuana originates, cannabis sativa, has been having a rebirth in popularity in Canadian society. There are a growing number of people who believe marihuana should be decriminalized much the same way alcohol was so many years ago. The reason people want to decriminalize it is for its possible therapeutic benefits that are just now starting to be explored and examined through science and research. As a result of this, it is now possible via special permission from the government, to obtain a certificate allowing an individual to legally possess marihuana for its use as a potential therapeutic agent:

> On July 30, 2001, the Narcotic Control Regulations was amended and the Marihuana Medical Access Regulations came into force. These regulations established a compassionate framework to allow the use of marihuana by people who are suffering from serious illnesses and where the use of marihuana is expected to have some medical benefit that outweighs the risk of its use.
>
> Under the new regulations, those who fall into one of two categories can apply for an Authorization to Possess marihuana for medical purposes. Holders of this authorization may possess a maximum 30-day treatment supply of marihuana at any given time.
>
> Category 1: This category is comprised of any symptoms treated within the context of providing compassionate end-of-life care; or the symptoms associated with the specified medical conditions listed in the schedule to the Regulations, namely:
>
> - Multiple Sclerosis: severe pain and/or persistent muscle spasms
> - Spinal Cord Injury: severe pain and/or persistent muscle spasms
> - Spinal Cord Disease: severe pain and/or persistent muscle spasms
> - Cancer: severe pain, cachexia, anorexia, weight loss, and/or severe nausea
> - AIDS/HIV infection: severe pain, cachexia, anorexia, weight loss, and/or severe nausea
> - Severe forms of Arthritis: severe pain
> - Epilepsy: seizures
>
> Category 2: This category is for applicants who have debilitating symptom (s) of medical condition (s), other than those described in Category 1. Under Category 2, persons with debilitating symptoms

> can apply to obtain an Authorization to Possess dried marihuana for medical purposes, if a specialist confirms the diagnosis and that conventional treatments have failed or judged inappropriate to relieve symptoms of the medical condition. While an assessment of the applicant's case by a specialist is required, the treating physician, whether or not a specialist, can sign the medical declaration.
>
> (Health Canada, Office of Cannabis Medical Access, 28/02/06)

As one can see, this type of regulation clearly has put marihuana back on the research hot list indicating that appropriate therapeutic protocols need to be established and determined. The exact mechanisms or mode of action of marihuana in treating or alleviating symptoms from any of the above conditions are not clearly understood but may relate to the endogenous cannabinoid receptors which have been recently identified and studied.

In order to obtain marihuana legally for medical use, an individual must apply for a permit. Health Canada requires some or all of the following forms to be completed:

Form A: Application for the Authorization to Possess Marihuana

Form B: (B1,B2,B3) These are the medical support forms that are filled out by the patient's physician(s), doctor(s) and/or specialist(s).

Form C: Application for Licence to Produce Marihuana by Applicant (for growing ones self)

Form D: Application for Licence to Produce Marihuana by Designated Person (to be completed by the person growing marihuana)

Form E: Consent of Property Owner to grow marihuana (for renters)

If the individual only wishes to possess marihuana for medical purposes, form A and B must be completed. If the individual is applying to both possess and grow his/her own marihuana for medical purposes, forms A, B, C, (and E, if applicable) must be completed.

If the individual is applying to possess dried marihuana for medical purposes and would like another person to grow the marihuana for him/her, forms A, B, D (and E if applicable) must be completed. The forms are available from Health Canada at: http://www.hc-sc.gc.ca/dhp-mps/marihuana/index_e.htmL

1.2 Cannabinoid Pharmacology

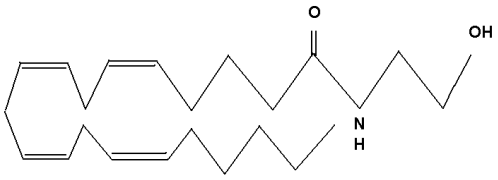
In human's, two cannabinoid receptors have been identified, CB1 and CB2 receptors. CB1 receptors are found mainly on neurons in the brain, spinal cord and peripheral nervous system, but are also present in certain peripheral organs and tissues, among them endocrine glands, leucocytes, spleen, heart and parts of the reproductive, urinary and gastrointestinal tracts (Pertwee, 1997). CB2 receptors are found mostly in areas related to the immune system, in particular, leucocytes, spleen and tonsils (Pertwee, 2002) (Gallieque, 1995). Activation of the CB1 receptor produces the psychotropic effects that are normally attributable to the "high" feeling often associated with the recreational use of marihuana in human. The major cannabinoid that binds to the CB1 and CB2 receptors is Δ^9-tetrahydrocannabinol (THC). Beyond the CNS effects of THC it can also induce tachycardia, increase cardiac output and can produce peripheral vasodilation (Perez, 1999). It is unclear the exact role the CB2 receptor

subtype plays although it may play a role in analgesia, anti-inflammatory and antineoplastic actions (Sanchez, 2001).

More recently, there is evidence suggesting that cannabinoids can modulate synaptic transmission, the cardiovascular system, and the immune system through receptors distinct from CB1 and CB2. There may be an additional "independent" endocannabinoid signaling system that involves other “independent” endocannabinoids distinct from anandamide and 2-arachidonoyl-glycerol (Mackie, 2006).

1.3 Endocannabinoids

After identification of the CB1 and CB2 receptors, endogenous ligands were also discovered for these receptors (Devane, 1992, Sugiura, 1995). The most important ligand discovered was anandamide. Anandamide has moderate affinity for the CB1 receptor and is rapidly metabolized. It shares most of the pharmacological effects of THC (Devane, 1992). Anandamide can be inactivated in the brain via two pathways. It can be either enzymatically cleaved to arachidonic acid and ethanolamine or it can be inactivated via neuronal uptake (Marihuana and Medicine, 2000). In general, the affinity of anandamide for cannabinoid receptors is only ¼ to ½ that of THC (Pertwee, 1997). Interestingly, the structure of anandamide (see figure 1.1) is not very similar to THC. This observation is similar to the relationship noted for opiod compounds and the corresponding endogenous ligand known as endorphins. Other endocannabinoids have also been discovered: 2-arachidonyl-glycerol (2-AG), homo-γ-linolenylethanolamide and 7,10,13,16-docusatetranylethanolamide (Hanus, 1993, Mechoulam, 1995).



Anandamide

Figure 1.1 chemical structure of Anandamide

1.4 Therapeutic Benefits of THC

Marihuana has been recognized as a potential therapeutic agent in a number of disorders. Very few well controlled studies have been conducted regarding its therapeutic benefits. The reason for this is because it is difficult to study marihuana without having to deal with the legal ramifications due to its status as being an illicit drug. The inherent difficulty in carrying out well controlled studies with a natural product also impedes advancements in the therapeutic use and study in humans. Marihuana plant contains many different chemical entities in varying concentrations. To complicate matters, the concentration of each chemical entity varies between different plant samples.

One of the major effects of THC is analgesia. Therefore, it has been indicated for use in chronic pain type disorders. Clinical studies should be directed at pain patients for whom there is a demonstrated need for improved management and where the particular side effect profile of cannabinoids promises a clear benefit over current approaches. The following patient groups have been identified as potential target groups

for further clinical studies of cannabinoids in the treatment of pain (Marihuana and Medicine, 2000):

- Chemotherapy patients, especially those being treated for the mucositis, nausea, and anorexia.
- Postoperative pain patients (using cannabinoids as an opiod adjunct to determine whether nausea and vomiting from opiods is reduced).
- Patients with spinal cord injury, peripheral neuropathic pain, or central post stroke pain.
- Patients with chronic pain and insomnia.
- AIDS patients with cachexia, AIDS neuropathy, or any significant pain problem.

1.5 Evaluation of the Potential Therapeutic Use of Cannabinoids

Pain can be treated via a number of different approaches. The classic opiod painkillers such as morphine, fentanyl, codeine and oxycodone can have limited success in patients due to unwanted side effects such as nausea, constipation, potential addiction and tolerance. It is for this reason that cannabinoids could be very successful candidates as adjuncts to opiods in the treatment of pain. There have been no well designed studies on the use of cannabinoids in post operative pain so it is uncertain as to their value in treating this condition. There have been a number of studies undertaken for cannabinoid use in chronic pain. Unfortunately, all of the studies are small and poorly constructed in general. In a study by Campbell a group of 10 cancer patients with chronic pain received a placebo, 5, 10, 15 and 20 mg of THC. Each subject had no idea which dose they were given in each successive trial. The 15 and 20 mg doses produced significant

analgesia with no cases of nausea or vomiting commonly encountered when opiods are used to treat chronic pain (Campbell, 2001). In April 2005, Health Canada approved cannabis medicinal extract (CME) with the indication of adjunctive treatment for symptomatic relief of neuropathic pain in adults with multiple sclerosis. It is a sublingually dosed whole-plant extract that contains a 1:1 ratio of THC and CBD. Notcutt et al designed a double blind placebo controlled crossover study of 34 patients with chronic stable pain who were poorly responsive to other treatments. Patients selected their 2 worst symptoms on which to record daily pain ratings. Patients also titrated their CME dose from 1 to 8 sprays per dose. There were significant improvements in the ratings of the two self selected symptoms with CME (Notcutt, 2004). Unfortunately, interpretation of these results is somewhat complicated as there was no standard dose. However, an inference can be made that the CME helped with the 2 patient self selected pain symptoms compared to placebo.

There is a growing body of evidence that cannabinoids may actually play a role as anticancer agent(s). In vitro and in vivo studies have found that naturally occurring and synthetic cannabinoids have antineoplastic effects in mice given xenografts of lung carcinomas, gliomas, thyroid epitheliomas, lymphomas and skin carcinomas (Guzman, 2003). The exact mechanism of these anti-neoplastic effects is unknown.

Nausea and vomiting can be caused by a wide variety of conditions, such as viral illness, cancer, radiation exposure, medications, motion sickness and poisoning. The anti-emetic effect of THC can be considered weak. In one study, prochlorperazine and THC were used to treat chemotherapy induced emesis. The results were quite poor as both agents failed to stop vomiting in two thirds of patients (Frytek, 1979). In another study comparing THC to metoclopramide (a well known anti-emetic agent), complete

control of emesis occurred in 47% of those treated with metoclopramide and 13% in those treated with THC (Grala, 1984). After this study, the FDA approved the use of THC in an oral dosage form known as dronabinol as an agent suitable for chemotherapy induced emesis. New classes of anti-emetics (in particular the serotonin receptor antagonists) have become the gold standard for chemotherapy induced emesis. As a result, THC should be considered to have weak anti-emetic properties and its use is best reserved for adjunct therapy when other methods have been tried unsuccessfully.

Wasting syndrome in acquired immune deficiency syndrome (AIDS) patients is defined by the Center for Disease Control and Prevention as the involuntary loss of more than 10% of baseline average body weight in the presence of diarrhea or fever of more than 30 days that is not attributable to other disease processes (CDC, 1993). In two studies by Beal et al, it was demonstrated that dronabinol clearly helps to battle AIDS induced anorexia and this effect has now been approved by the FDA (Beal, 1995, 1997).

Other therapeutic benefits that are currently being explored are the ability of marihuana to help spasticity due to spinal cord injury and multiple sclerosis (Killestein, 2002). Much of the evidence stating that marihuana may be beneficial for treatment of multiple sclerosis is largely subjective involving small groups. Currently, a number of large-scale phase III clinical trials are under way to further elucidate the use of cannabinoids in the symptomatic treatment of multiple sclerosis.

It is also known that THC has a favourable effect on intraocular pressure (Crawford, 1979) which plays an important role in glaucoma treatment. Unfortunately, the more mainstream agents (timolol, latanoprost, brimonidine) used in treating glaucoma have greater efficacy since marihuana may leave the patient with the undesirable psychotropic effects of marihuana.

There has also been some evidence that marihuana may play a role in helping to alleviate seizures in epilepsy patients. (Gordon, 2001). This aspect of the drug is largely unstudied and more clinical trials would need to be carried out before any conclusions could be determined.

1.6 Cannabinoids Of Interest

A total of 66 phytocannabinoids have been identified. Most of the 66 identified fall into a number of subclasses or types: the canabigerol (CBG), cannabichromene (CBC), cannabidiol (CBD), Δ^9 – tetrahydrocannabinol (THC), cannabinol (CBN), Δ^8 – tetrahydrocannabinol, cannabicyclol, cannabielsoin, , cannabinodiol and cannabitriol types. (A total of nine cannabinoids belong to the Δ^9 – tetrahydrocannabinol group, with side chains of one, three, four and five carbons (Grotenherman, 2003). There are two numbering systems commonly used when it comes to numbering the carbon atoms in cannabinoid compounds. The first system is the monoterpene system (Figure 1.2) which was used and created as a consequence of the biosynthetic pathway in plants for the synthesis of cannabinoids.

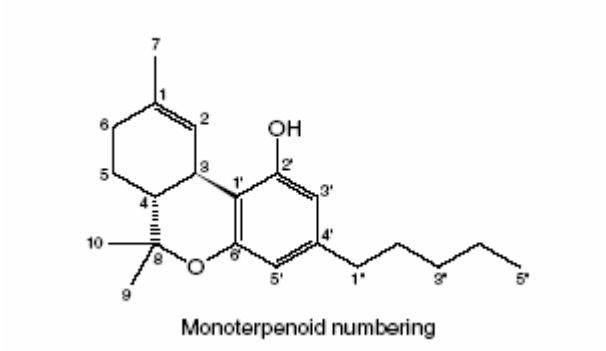


Figure 1.2 Monoterpenoid chemical numbering system

The second system is the dibenzopyran system based on IUPAC nomenclature and numbers the carbons in the following fashion (Figure 1.3):

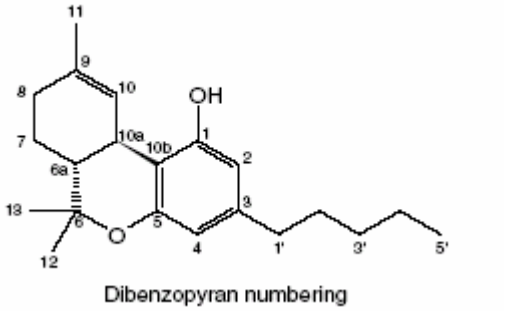
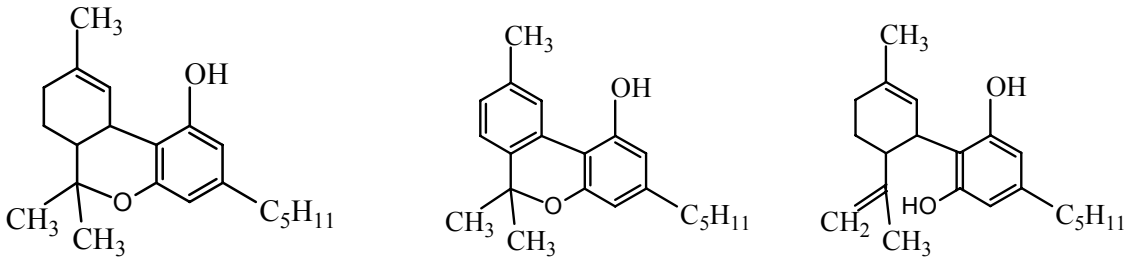


Figure 1.3 Dibenzopyran chemical numbering system

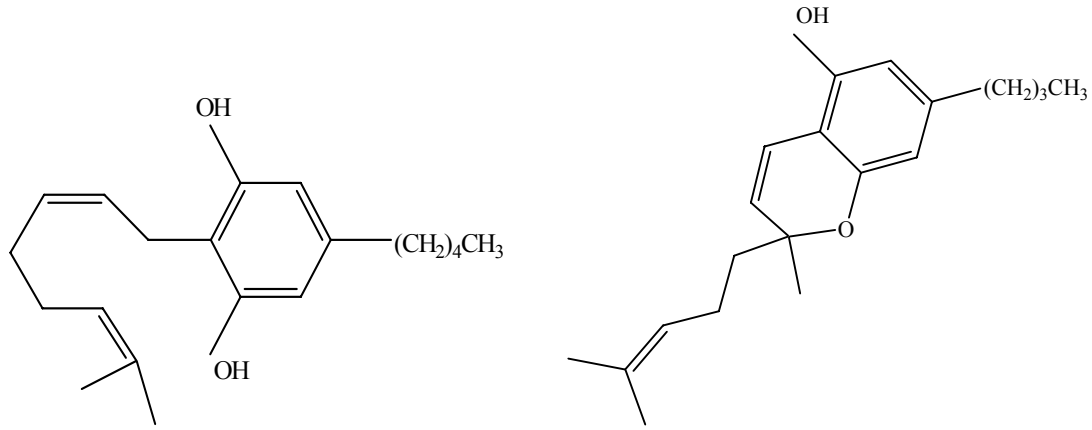
Throughout this paper, all structures will be referenced using the dibenzopyran system. As a result of the two numbering systems, it is important to realize that Δ^9 THC and Δ^1 THC are the same molecule that have been numbered using either numbering system. The majority of the literature uses the Δ^9 THC dibenzopyran nomenclature.

The cannabinoids found in greatest abundance in a typical marihuana plant sample are Δ^9 – tetrahydrocannabinol (THC), cannabidiol (CBD) and cannabinol (CBN) (Figure 1.4). As well, there are often small amounts of cannabichromene (CBC) and canabigerol (CBG) (Figure 1.5). The major most acidic cannabinoid found is Δ^9 - tetrahydrocannabinolic acid (THCA-A) (Figure 1.6).



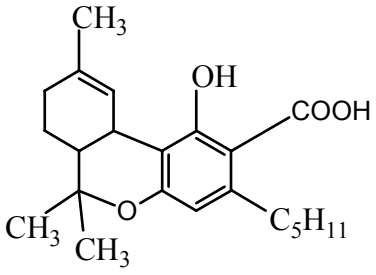
Δ^9- tetrahydrocannabinol (THC) Cannabinol (CBN) Cannabidiol (CBD)

Figure 1.4 Chemical structures of THC, CBN, and CBD



Cannabigerol (CBG) ***Cannabichromine (CBC)***

Figure 1.5 Chemical structures of cannabigerol (CBG) and cannabichromine (CBC)



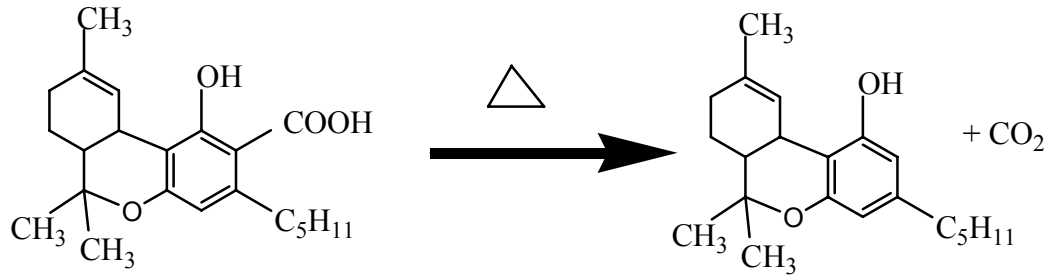
Δ^9- tetrahydrocannabinolic acid (THCA-A or THCA)

Figure 1.6 Chemical structure of THCA-A

Acidic cannabinoids are the major constituents in cannabis sativa plant with high concentrations found in the leaves and buds; however they readily undergo decarboxylation to their respective neutral cannabinoids upon mild to extreme heating. THCA is pharmacologically inactive; however after it is decarboxylated it forms the pharmacologically active THC (Nova Institute: THC-Limits for food part 2, 2002) (Figure 1.7). THCA exists as two geometric isomers THCA-A and THCA-B differing only in the location of the carboxylic acid group in either the 2(THC-A) or 4(THCA-B) positions.

Cannabidiol (CBD) is a nonpsychotropic cannabinoid, for which sedating, antiepileptic, antidystonic, antiemetic and anti-inflammatory effects have been observed. It reduces intraocular pressure, is neuroprotective and antagonizes the psychotropic and several other effects of THC. Anxiolytic and antipsychotic properties might prove useful in psychiatry (Grotenhermen, 2003).

The nonpsychotropic cannabinoids CBG and CBC show sedative effects. CBG has been observed to decrease intraocular pressure, show antitumour activity against human cancer cells and has antibiotic properties (Grotenhermen, 2003).



Δ⁹- tetrahydrocannabinolic acid ***Δ⁹- tetrahydrocannabinol***

Figure 1.7 Decarboxylation of THCA to THC

THC is considered to be the source of the main pharmacological effects observed after the consumption of cannabis. This includes the marihuana-like "high" action and at least part of the medicinal properties of the plant. Cannabinoids exert many effects through the activation of G protein coupled cannabinoid receptors in the brain and peripheral tissues (Figure 1.8).

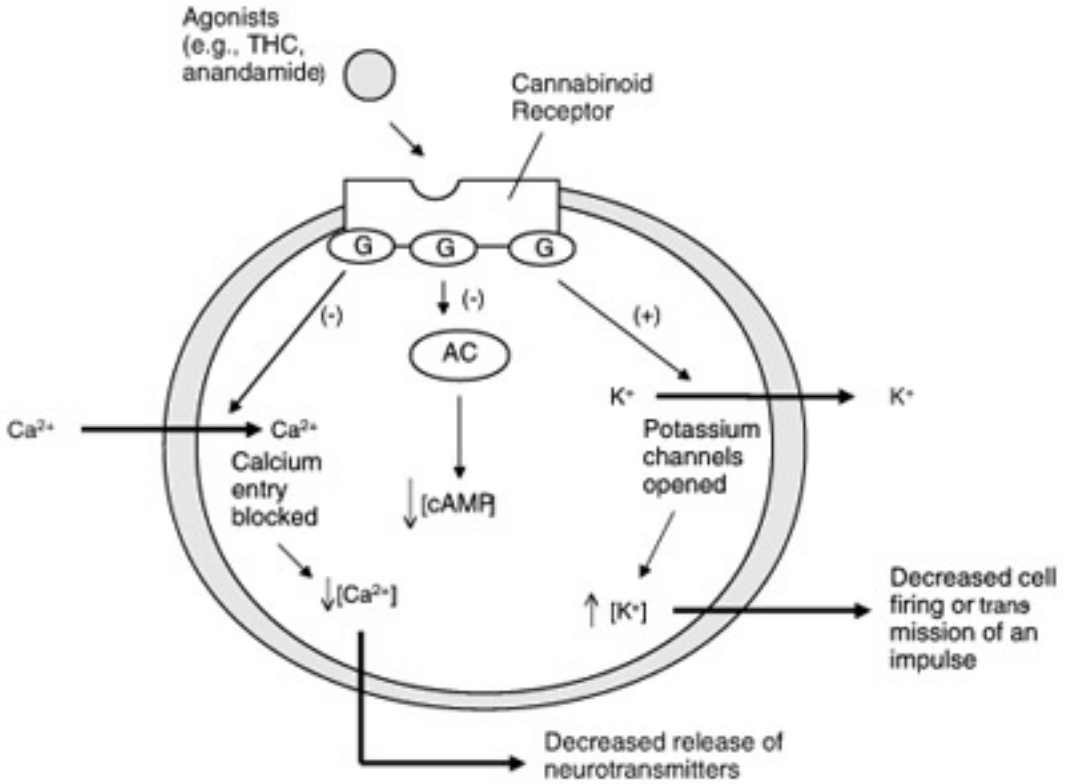


Figure 1.8 Cannabinoid agonists trigger a series of reactions within cells. Cannabinoid receptors are embedded in the cell membrane, where they are coupled to G proteins (G) and the enzyme adenylyl cyclase (AC). Receptors are activated when they bind to ligands, such as anandamide or THC in this case. This triggers a variety of reactions, including inhibition (—) of AC, which decreases the production of cAMP and cellular activities dependent on cAMP; opening of potassium (K^+) channels, which decreases cell firing; and closing of calcium (Ca^{2+}) channels, which decreases the release of neurotransmitters. Each of those changes can influence cellular communication. (copied with permission from Marihuana and Medicine: Asessing the Science Base, 1999, Institute of Medicine (IOM), USA)

Additionally, there is evidence of non-receptor dependent mechanisms. For example, neuroprotective effects in ischemia and hypoxia (Hampson, 2002), effects on the immune system, and some effects on circulation (Ralevic, 2002).

1.7 Pharmacokinetics of THC

Cannabis plant is usually administered via inhalation or taken orally. It is inhaled by smoking a marihuana cigarette or taken orally as capsules or in baked foods or liquids. THC is detectable in plasma almost immediately after smoking due to the fast permeability across the lung mucosa (Huestis, 1992). Peak plasma concentrations occur 3-10 minutes after onset of smoking. Psychotropic effects begin immediately, reach a maximum after 15-30 minutes and taper off within 2-3 hours (Grotenherman, 2003). Systemic bioavailability generally ranges between 10-35% (Lindgren, 1981).

Oral administration results in slow and erratic absorption with maximal plasma concentrations usually seen after 1-2 hours (Sporkert, 2001). Extensive first pass metabolism results in low bioavailability compared to the initial dose. Psychotropic effects set in with a delay of 30-90 minutes and reach their maximum after 2-3 hours and last for about 4-12 hours depending on dose (Grotenherman, 2003).

1.8 Metabolites

THC is metabolized in the liver by microsomal hydroxylation and oxidation via the CYP450 system. A member of the CYP2C subfamily of isoenzymes plays the major role for its metabolism in humans (Watanabe, 1995). The 11^{th} carbon on the THC molecule is the major site of metabolic attack. The two major metabolites of THC are 11-hydroxy-delta-9-THC and 11-nor-9-carboxy-delta-9-THC (Niveau, 2002) (see Figure

1.9). Hydroxylation of the 11th carbon followed by oxidation to the acid moiety allows for glucuronidation to occur. THC is excreted within days and weeks, mainly as acid metabolites. Approximately one third of THC metabolites are excreted in the urine and about 65-80% is eliminated in the feces. Less than 5% of an oral dose of THC is not metabolized and is eliminated in the feces (Wall, 1983).

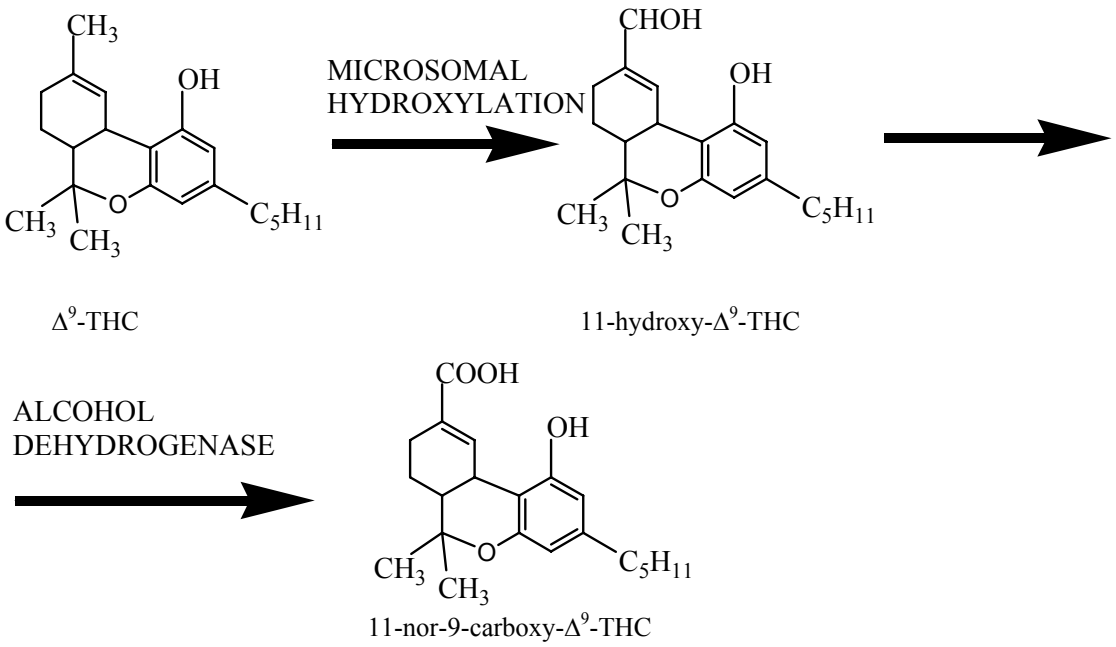


Figure 1.9 Metabolism of THC in the liver and lungs

1.9 Mainstream Smoke

A common method of taking or using marihuana is by the inhalation route. Very little research has been carried out on determining cannabinoid profiles or relative concentration of cannabinoids in the smoke resultant from the combustion of plant material. In this regard, a key question relates to whether there is a difference in the

cannabinoid profile of cannabis sativa plant before and after combustion. In fact, throughout the literature, it is assumed that the total of the THC and its corresponding acids is almost always considered in potency content. However, very few reports acknowledge that the conversion of THCA to THC is not a 100% quantitative. Therefore, throughout the literature it has been assumed that however much THCA and THC is in the plant is the total amount that is delivered in the smoke. This is likely an oversimplification. Recently a study by Dussy et al showed that maximal conversion in a optimized analytical equipment setup yields about 70% conversion from THCA to THC. Furthermore, in the simulation of the actual smoking process, only about 30% of the spiked THCA substance could be recovered as THC (Dussy, 2004).

1.10 Sidestream Smoke

Interest in sidestream smoke or passive inhalation of combusted plant material primarily arises as a consequence of litigation. The argument that the cannabinoids found in an individual's urine arose due to the person's presence in an area where marihuana was being smoked was typified by the 1998 winter Olympics incident. In 1998, there was a Canadian athlete in the Winter Olympic Games who was stripped of his medal for testing positive for THC. It was argued that the individual never in fact smoked any marihuana but had been exposed to it via sidestream smoke. In the end, the person was allowed to keep the medal as the sidestream smoke argument held up. However, with that being said in one study, 5 drug free volunteers were passively exposed to the sidestream smoke of 4 and 16 marihuana cigarettes containing 2.8% THC for one hour each day for six consecutive days. The room size in which they were exposed to the cannabis smoke had dimensions of 2.1m x 2.5m x 2.4m which is a

volume of roughly 12,225 liters. The study showed that significant amounts of THC were absorbed by all subjects exposed to the 16 cigarette setup. At the 4 cigarette setup, the subjects tested positive only infrequently or were negative. Furthermore, in the 16 cigarette setup, the subjects complained that the smoke in the room was so thick that it would be impossible to not knowingly be inhaling the smoke when surrounded by such a thick cloud (Cone et al, 1986). This study was repeated two more times with nearly identical results (Cone et al, 1987). Therefore this would suggest that although sidestream smoke can produce measurable levels of cannabinoid in vivo the individual(s) would consciously know they were in a room with cannabis smoke. Furthermore, conducting oral fluid testing for presence of THC in passive versus active subjects clearly shows a direct relationship between oral fluid concentrations and urine analysis in an active smoker versus a passive smoker (Niedbala, 2005).

1.11 Methods Used in the Chemical Analysis of Cannabis

In the past, the vast majority of cannabinoid analytical studies have been carried out using gas chromatography (GC) as the primary means to separate the cannabinoid compounds. This method relies upon the volatility of the various cannabinoids and readily converts the non-volatile carboxylic acids to their respective volatile counterparts. However, it is also important to realize that cannabinoids are very heat labile compounds, in particular the acid entities. As a result, it is extremely difficult to get a true measurement of the cannabinoids in a plant sample without thermally decomposing apriori or derivatizing the sample so as to protect the thermally labile acids. This is more readily accepted if you are working with one compound; however,

derivatizing a plant sample that contains numerous cannabinoid compounds makes derivatization a poor choice because you do not know what exactly is being derivatized or whether the derivitization is quantitative. There are some 421 identified compounds in cannabis, 66 of which are cannabinoids (Turner, 1982). Unfortunately, the research community continues to use GC as a means to qualitatively and quantitatively characterize cannabinoids. The primary reason for this is because derivatizing using GC has been carried out so frequently that it is an accepted method.

A method by LeBelle and Savard named "Gas Chromatographic Determination of Tetrahydrocannabinol in Cannabis" is one example of many of the flaws mentioned above. This method uses GC-FID as a means to quantify the THC content in cannabis plant material. The first problem is it is assumed that THCA is found in small quantities in cannabis when in fact it is usually in the largest abundance of all cannabinoids. The second problem is there is no mention of any derivitization which means the cannabinoids are being injected onto a 200°C column with a temperature program up to 290°C in 8 minutes. This will most certainly lead to thermal destruction of the cannabinoids. Third, the way in which the standards of THCA and THC are used in order to calculate the percent conversion of THCA to THC is severely flawed. The way the percent conversion is calculated is by injecting a known amount of THC on the column and then a known amount of THCA on the column and then taking the ratio of peak areas. It is stated that the conversion should be 90% or greater for THCA to THC but the larger problem here is no account is made for the thermal destruction of both compounds to unknown quantities. Thermal destruction in underivatized samples is a very big problem and this method of GC quantification and analysis is essentially useless with cannabinoid compounds without derivatization. In a recent study by Dussy

et al, they determined that maximal conversion from THCA to THC inside a GC system to be approximately 67% at an injector temperature of 220 °C with the remainder of the products being lost to thermal destruction and the creation of polymeric material (Dussy, 2004). It has not been until more recently that people are starting to use HPLC as a means to separate and quantify the cannabinoids.

1.12 High Performance Liquid Chromatography Instrumentation in Marihuana Analysis

High performance liquid chromatography (HPLC) is becoming more commonly used for marihuana analysis. A major advantage of HPLC over gas chromatography in cannabinoid analysis is that acidic cannabinoids can be detected which is not the case in gas chromatography. A method developed by Dautbegovic et al in 2002, is presented to illustrate HPLC instrumentation. A solvent pump, an injector, variable-wavelength ultraviolet (UV) detector set at 230 nm, a column (4.6 x 150 mm), and data processor make up the HPLC instrument used in this particular analysis. A flow rate is set at 1.5 mL/min and mobile phase consists of 65 % acetonitrile, 35 % water with 0.03% formic acid.

The mobile phase, column, flow rate and a detector can be varied depending on desired specificity and selectivity for the analytes in question. Therefore, the HPLC system may be modified to allow for separation of numerous analytes.

1.13 The Devices Used for Combusting and Inhaling

The combustion and inhalation of marihuana smoke can be accomplished in a number of ways. The most common practice is to “roll” plant material into a cigarette

and smoke it in a manner similar to a tobacco cigarette. Alternatively, pipes, water bongs and vaporizers are also used to combust the marihuana material. The end result is the same in that marihuana is converted from a solid to a gaseous state and the gas is inhaled into the lungs by an individual. Some find water bongs or vaporizers are more comfortable as the smoke is less irritating to the mucosa lining the respiratory tract and the mouth.

2 RATIONALE AND OBJECTIVES OF THE STUDY

This project was undertaken to examine cannabinoid content in combusted plant material obtained through the Health Canada medicinal marihuana initiative. In order to carry out this project, it was necessary to develop a reliable combustion method and technology to trap and extract cannabinoids from the smoke. This work is deemed essential in order to evaluate the dosing of subjects using cannabis since the most common way of ingesting marihuana in people is by smoking. To the best of our knowledge, no work has ever been done on the actual THC content of marihuana smoke. It is known that by burning cannabis plant material, much of the THCA found in the plant is converted to THC which is the pharmacologically active stupefacient ingredient in cannabis whereas the THCA is considered a "pro-drug". It is hoped that by analyzing the smoke of combusted cannabis plant tissue extract, similar chemobotanical profiling data as that currently available for uncombusted plant tissue can be obtained. In the plant tissue, there are other compounds in cannabis extract (CBD, CBN, etc) which may also be affected by heat. These compounds could also display important pharmacological activity. It is hoped that if a reliable, reproducible model of quantifying the smoke can be established, these results could be correlated with the compounds found in the cannabis plant extract and a relationship could be established.

Thus it is possible to summarize the research proposal as follows:

The hypothesis of this research is can qualitative and quantitative changes be analyzed and measured from the combustion products of cannabis plant tissue. More specifically,

upon the combustion of marihuana plant tissue is the conversion of THCA to THC 100% quantitative and furthermore, are there qualitative and or quantitative conversions of cannabinoids in the plant after combustion?

Therefore, in summary the major objective required in this project are the following

- Creation of a method to reliably capture and sample smoke produced from burning cannabis plant material.
- Use both an HPLC-UV and LC-MS-MS method to both qualitatively and quantitatively measure the components found in the cannabis smoke.
- Apply the above methods to a standardized sample of cannabis plant material.

3 EXPERIMENTAL

3.1 Chemicals and Reagents

Table 3.1 lists the various chemicals and reagents used in the work described in the thesis. The name, grade, and source of each chemical and reagent are identified in the table. All chemicals and reagents were used without further purification or processing.

Table 3.1 Table of chemicals and reagents used in the thesis

Name of the chemical/reagent	Grade	Source
Methanol	Omnisolve	EM Science
Acetonitrile	HPLC	EM Science
Water	RO de-ionized	Bronstead/Thermodyne Nanopure® water system
Formic acid	98-100%	EM Science
Nitrogen gas	Commercial	Praxair, Saskatoon, SK

All EM Science chemicals and reagents were purchased from VWR in Edmonton, Alberta.

3.2 Instrumentation

The HPLC instrumentation was composed of a Millipore Waters Model 590 pump, Millipore Waters Lambda Max 480 spectrophotometer set at 230 nm, C8 reverse phase column (4.6mmX150mm, Zorbax), and Shimadzu SIL-9A autoinjector. Data acquisition and processing were automated through the use of Waters Empower chromatography software. The pump, spectrophotometer, column, and chromatographic software were purchased from Waters in Mississauga, Ontario. The auto-injector was purchased from Mandel in Guelph, Ontario.

Table 3.2 lists the various pieces of instrumentation used in processing and handling of the samples described in the thesis. The names of the instrumentation, the source, and addresses of the suppliers are indicated.

Table 3.2 Table of instrumentation used in the processing and handling of the samples

Name of the Instrument	Source	Address
IKA-Vibrax-VXR shaker	VWR	Edmonton, AB
Laboratory refrigerator set at 4°C with a separate freezer compartment	VWR	Edmonton, AB
15mL disposable borosilicate glass culture tubes (size: 16 x 125mm)	VWR	Edmonton, AB
Polypropylene stoppers (size: 16mm)	Canadawide Scientific	Ottawa, ON
Eppendorf standard tube, 500 microcentrifuge tubes natural polypropylene (1.5mL)	VWR	Edmonton, AB
Kimble septa 10” PTFE	VWR	Edmonton, AB
Kimble insert vial, clear (0.3mL, 8mm)	VWR	Edmonton, AB
Chromacol crimp top caps with Teflon/rubber septum	VWR	Edmonton, AB

Pasteur Pipet	VWR	Edmonton, AB
Eppendorf 2-20uL pipettor using Biorad BR-41 pipet tips	BioRad	Mississauga, ON
Eppendorf 20-200uL pipettor using Biorad BR-41 pipet tips	BioRad	Mississauga, ON
Eppendorf 100-1000uL pipettor using Biorad BR-39 pipet tips	BioRad	Mississauga, ON
Eppendorf repeater pipet using Eppendorf 2.5mL combitips plus	VWR	Edmonton, AB
Hamilton gas tight syringes (500 μL)	VWR	Edmonton,AB
Kimble 1.8mL Robo® vial amber (12x32 mm) with Teflon/rubber septum	VWR	Edmonton,AB
Kimble 20mL glass scintillation vials with Teflon screw caps	VWR	Edmonton,AB
Pyrex 2000 mL glass bottle	VWR	Edmonton,AB
Kimax-35 1000 mL glass bottle	VWR	Edmonton,AB
Kimax 1000mL graduate cylinder	VWR	Edmonton,AB
Kimax 2000mL graduate cylinder	VWR	Edmonton,AB
Pyrex 1000mL filter flask	VWR	Edmonton,AB
MFS Micro Filter System	VWR	Edmonton,AB
PALL Nylaflo® nylon membrane filter (0.2μm, 47mm)	VWR	Edmonton,AB
Cole Parmer Digi-sense thermometer Type K Thermocouple	Cole Parmer	Missisauga, ON
Gas sampling bulb	Cole Parmer	Missisauga, ON
Savant 190 Vacuum Pump	Savant	Missisauga, ON
3M solid phase extraction cartridge size 3mm x 7mL	3M	Missisauga,ON

Isolute solid phase extraction cartridge size 3mm x 7 mL	VWR	Missisauga,ON

3.3 Solutions

3.3.1 HPLC Mobile Phase Solution

HPLC mobile phase solution consisted of 65 % acetonitrile and 35 % of aqueous 0.12 % formic acid solution. The HPLC mobile phase solution was prepared by measuring 700 mL deionized reverse osmosis (RO) water in a graduate cylinder, which was then transferred into a filter flask and 2.38 mL of formic acid was added to the water. 1300 mL of acetonitrile was measured in a graduate cylinder and added to the 702.38 mL of the above prepared aqueous solution. The mobile phase was then filtered and degassed in a micro filter system (0.2 μm filter). Mobile phase was stored in a 2000 mL glass bottle at room temperature. Additional mobile phase was prepared as required for the experiments performed in the thesis. New mobile phase was prepared weekly or within a shorter time frame.

3.3.2 HPLC Rinse Solution

The HPLC rinse solution used in the thesis consisted of 65 % acetonitrile and 35 % RO water. The HPLC rinse solution was prepared by measuring 350 mL of RO water in a graduate cylinder, which was then transferred into a filter flask. A 650 mL of acetonitrile was measured in a graduate cylinder and added to the water in the filter flask. The HPLC rinse solution was then filtered and degassed in a micro filter system (0.2 μm filter). The solution was stored in a 1000 mL glass bottle at room temperature.

Additional rinse solutions were prepared as required for the experiments performed in the thesis.

3.3.3 THCA, THC, CBD, and CBN Standard Stock Solution

Standard solutions required in the thesis were THC methanol solution purchased from Cerilliant, CBN methanol solution and CBD methanol solution, both purchased from the Sigma Aldrich. THCA in methanol was obtained in house by preparative isolation from plant material as described below. All standard solutions were 1 mg/mL concentrations.

A combined stock solution of THCA, THC, CBD, and CBN was prepared from 1mg/mL standard methanol solutions. Exactly 1 mL of each standard was combined into a 15 mL culture tube and taken to dryness at 35^{o}C with the aid of a gentle stream of nitrogen. The dried residue was reconstituted in 1 mL of methanol to give a combined stock solution with a concentration of 1 mg/mL for each compound. This solution was stored at 4^{o}C and used to prepare calibration standards as detailed below. Additional standard stock solutions were prepared as required for the experiments performed in the thesis.

3.3.4 Collection Of THCA-A

THCA-A was collected from marihuana plant extracts since no commercial source of THCA was available. The THCA solution was prepared using a method by Dautbegovic and Zhang. The method is as follows: Several marihuana plant methanolic extracts were combined to give approximately 100 mL total volume of extracts. Methanol was evaporated at 35°C under a gentle stream of nitrogen. Residue that

remained at the bottom of a test tube was reconstituted in 10 mL of methanol. The methanol solution was then injected onto HPLC and a peak corresponding to THCA-A was collected into a glass scintillation vial. The collected solution was then evaporated at 35°C under gentle stream of nitrogen. The residue remaining in a test tube was weighed and enough methanol was added to make 1 mg/mL THCA-A standard solution in methanol. The THCA-A solution was shown to be 88-90% pure and was stored at 4°C until further use. Purity was assessed by HPLC-UV where the total area for THCA was divided by the total area of all observed peaks.

3.3.5 Working Solutions For THCA, THC, CBD and CBN Standard Curves

The working solutions for THCA, THC, CBD, and CBN standard curve were prepared from THCA, THC, CBD, and CBN standard stock solution as described in section 3.3.3 The working solutions were prepared in Eppendorf 500 microcentrifuge tubes (1.5 mL vials). Table 3.3 outlines the preparation of working solutions of THC, CBD, and CBN.

Table 3.3 Preparation of the THCA,THC, CBD, and CBN working solutions for the standard curve

Calibrator concentration	Volume of standard stock solution	Volume of methanol
200 μg/mL	100 μL of 1 mg/mL	400 μL
100 μg/mL	200 μL of 200μg/mL	200 μL
50 μg/mL	200 μL of 100μg/mL	200 μL
25 μg/mL	200 μL of 50μg/mL	200 μL
12.5 μg/mL	1000 μL of 25 μg/mL	200 μL

From each of the above working solutions, 75 μL was measured and placed in the microcentrifuge tube vials. The unused portion of working solutions of THCA, THC CBD, and CBN were stored in the autosampler Robo® vials at 4°C. Additional working solutions of THCA, THC, CBD, and CBN were prepared as required for the experiments in the thesis.

3.3.6 Quality Control THCA, THC, CBD, and CBN Solutions

The quality control (QC) solutions for THCA, THC, CBD, and CBN were prepared from THCA, THC, CBD, and CBN standard stock solution as described in section 3.3.3. The quality control solutions were prepared in Eppendorf 500 microcentrifuge tubes (1.5 mL vials). Table 3.4 outlines the preparation of quality control solutions of THCA, THC, CBD, and CBN.

Table 3.4 Preparation of the THCA,THC, CBD, and CBN quality control solutions

Quality Control concentration	Volume of standard stock solution	Volume of methanol
150 μg/mL	75 μL of 1 mg/mL	425 μL
75 μg/mL	200 μL of 150 μg/mL	200 μL
37.5 μg/mL	200 μL of 75μg/mL	200 μL

Quality control solutions were stored in autosampler, Robo® vials with 400 μL of solution in each vial. Quality control solutions were stored at 4°C and additional quality control solutions were prepared as required for experiments in the thesis.

On the day of the experiment, one vial of each quality control solution was removed from the fridge and 75 μL of each quality control was placed in the Eppendorf 500 microcentrifuge tubes (1.5 mL vials).

3.3.7 THCA-A Purity Check

THCA-A collected from marihuana plant extracts (see section 3.3.4 for details) was evaluated for purity of THCA-A. In order to assess the purity of the sample, 20 μL of THCA-A 1 mg/mL stock solution (see section 3.3.3) was injected onto the HPLC. The chromatogram that was produced showed several small peaks and a major peak representing THCA-A. Areas under all peaks in the chromatogram were added together. The areas under all peaks added together divided the area under the THCA-A peak. The result was then multiplied by one hundred in order to get percentage of THCA-A purity. In all samples, the purity was between 88-90%. The area under the curve of THCA is 1.1 times greater consistently than the area of THC for same given concentration. This means that THCA gives a slightly stronger response by the UV detector then THC.

3.3.8 Precision and Accuracy

Precision and accuracy were evaluated by analyzing standards and quality controls in replicates of five or ten depending on the experiment. Standards were made as per section 3.3.3 and analyzed in replicates of two with every new experiment. Quality control samples were analyzed in replicates of two in every experiment to further demonstrate accuracy and precision of the HPLC method.

3.4 Analytical Method

3.4.1 Extraction of Marihuana Material

The contents of each plant material sample (approximately 5 g) were ground to a fine homogeneous powder using a coffee bean grinder (ground at full speed for 1 minute followed by a meticulous cleaning of the grinder using a combination of water and methanol). A representative sample of approximately 5 mg of plant material (weight was recorded accurately) of each plant sample was weighed in a 15 mL disposable glass tube. Methanol (3mL) was added to each sample, the glass tube containing plant sample and methanol was capped and placed on IKA-Vibrax shaker at speed 1000. The samples were shaken for 30 minutes after which methanol was removed to a 20 mL scintillation vial and capped. The procedure was repeated one more time for each unknown sample resulting in 6 mL total of methanol extract in each instance. The unknown samples were stored at 4^{o} C until analysis.

On the day of the analysis, 50 μL of each unknown was placed into 1.5 mL microcentrifuge tube vials and were injected onto the HPLC.

3.5 Combustion Of Marihuana Material

3.5.1 Pipe Method

A pipe was attached to the 3M SPE cartridge that was then attached to a vacuum pump (see figure 4.3). The 5 mg sample was then placed in the mesh in the pipe and the vacuum was switched on. The cannabis was then ignited using a butane lighter and combusted to a white ash. The SPE cartridge was extracted using 1 mL of methanol and

the extract was analyzed on HPLC-UV to determine the cannabinoids profile and recovery from the original sample. This setup worked very well as large amounts of THC were seen on the HPLC-UV as well as quantifiable amounts of CBD, CBN and CBC.

3.5.2 Test Tube Method

A 5 mg sample of marihuana material was weighed and stored in capped test tubes until needed. When it was time to analyze the sample, it was transferred to the smoking device and combusted. This machine worked by placing the sample to be ignited in the bottom of a test tube (see figure 4.4). A small piece of flexible hose was then attached to the test tube that had an SPE cartridge attached to it. The vacuum pump was then attached to the base of the SPE cartridge. The sample was heated using a Bunsen burner or butane lighter. Combustion could clearly be seen as smoke began to form inside the test tube. At this point, the vacuum was turned on and the smoke was pulled out of the test tube and deposited on the SPE cartridge. Once the sample showed only ash remaining, the vacuum was turned off. The SPE cartridge was then extracted with 1 mL of methanol on the vacuum extraction manifold. At the same time, the test tube had 1 mL of methanol added to it and was capped and then shaken on a vibrax shaker at speed 1000 for 10 minutes. After completion of shaking, the sample was filtered and placed in storage tube so it could be analyzed. Each extract was then analyzed and the total amounts of each analyte found in the test tube and the SPE cartridge were added together to give the total cannabinoid profile.

3.5.3 Oven Method

A sample of cannabis plant with a known concentration of THCA was placed inside a glass test tube and then capped with a heat stable screw cap. The experiments were then performed at varying temperatures and time duration. The test tubes were then removed from the oven and allowed to cool. After cooling, 3 mL of methanol was added to each test tube and shaken on a vibrax shaker speed 1000 for ten minutes. The methanol extract was then filtered and placed inside a suitable vial that could then be analyzed under HPLC-UV and/or HPLC-MS/MS.

3.5.4 Measurement of Temperature of Marihuana Cigarette

A sample of cannabis plant was placed inside one of the pipes and the temperature of the lit portion of marihuana was measured using a Cole Parmer Thermosense digital thermometer and probe.

3.5.5 Measurement of Vacuum Used to Simulate Drawing of Combusted Marihuana Smoke

A vacuum gauge was used to record the vacuum at which the air was being drawn through the smoking machine's orifice.

3.6 HPLC Analytical Method

HPLC consisted of the parts described in section 3.2.0 and was maintained as required throughout the experimental time for the thesis. The column was installed and conditioned according to the manufacturers instructions. Mobile phase was prepared as described in detail in section 3.3.1 and set at isocratic flow rate of 1.5 mL/min. All

experiments were performed at room temperature. The length of analysis of each individual sample was twenty minutes. The UV detector was set at a wavelength of 230nm. All injections were performed by the autosampler and were set at 20 μL. Data were acquired and processed by the peak integrations using the Waters Empower chromatography software. All necessary dilution factors were applied in order to report the concentrations of each analyte in terms of gram percent taking into account the original weight of tissue extracted.

3.7 LC-MS-MS Analytical Method

For the liquid chromatography tandem mass spectrometry (LC-MS-MS) method, the column was installed and conditioned according to the manufacturers instructions. The column used was XTerra® MSC_{18} 3.5 μm (2.1mm x 100 mm). The mobile phase consisted of 70 % acetonitrile with 30 % aqueous 0.1 % formic acid solution. The flow rate was set at 0.25mL/min. The detector used for the LC-MS-MS method was Micromass Quatro Ultima tandem mass spectrometer. The software version was MassLynx 3.5. All injections were performed by the auto-sampler and were set at 5 μL. The positive ion model used for THC, CBD, CBN, CBC, CBG, and THCA-A is outlined in table 3.5.

Table 3.5 Positive ion model used for THC, CBD, CBN, CBC, CBG, and THCA-A

Analyte	Parent ion (m/z)	Daughter ion (m/z)	D well (sec.)	Cone (E lab)	Collision energy (E lab)	Retention (min)
CBN	311.4	223.2	0.3	30	20	9.31
THC	315.2	193.2	0.3	30	20	11.82
CBC	315.2	193.2	0.3	30	20	16.14
CBD	315.3	123.3	0.3	30	30	5.73
CBG	317.4	193.2	0.3	30	15	5.48
THCA-A	359.4	219.2	0.3	30	30	16.95

The peak integrations were acquired and processed using the Waters Empower chromatography software. All the necessary dilution factors were applied to report the concentration of each analyte in terms of gram percent taking into account the original weight of tissue extracted

4 RESULTS AND DISCUSSION

4.1 Smoking Machine Analysis

4.1.1 Background

The cannabinoid profile of combusted cannabis is important since it more truly represents the components available for absorption in human. To date, much of the analytical work carried out on cannabinoids has been done by GC and therefore the resulting profile may be considered to represent the thermally decomposed profile of plant tissue extract. Whether such a profile is identical to the chemical profile obtained during clandestine combustion such as that used in the delivery of marihuana is not clear. In this regard, the specific thermal conversion of THCA to THC has been considered quantitative during GC analysis but whether this is the case during clandestine combustion has not been reported. Therefore, an experiment was designed to examine both qualitatively and quantitatively the compounds found in marihuana plant tissue after combustion has taken place and furthermore, to compare these to the original chromatographic profile of the raw plant. Quantitative analysis of cannabis combustion smoke is fraught with some difficulties. First the system needs to mimic techniques used in the field and yet capture all of the smoke. A number of model devices were employed along with actual devices used in the field.

4.1.2 The Smoking Machines

Four different smoking machines were employed. Each of these is discussed in the following sections with a brief comment related to the apparent suitability of each set up. For those machines that worked well, a more in depth study was undertaken.

4.1.2.1 Smoking Machine #1

The first smoking machine followed the preconceived idea that if you ignited the sample and then pulled the smoke through a suitable solvent such as methanol, the cannabinoids would dissolve in the methanol and the methanol could be analyzed using HPLC-UV. To test this technique a vaporizer device was employed (see figure 4.1). This device represents a closed system that has a controllable intake and exhaust line. By connecting the exhaust line in such a way that all of the exhaust must bubble through a methanol trap, it should be possible to extract cannabinoids present in the smoke into methanol. A 50 mL syringe was used to draw smoke through the exhaust and into the trap (see figure 4.1). A chromatographic profile could then be obtained through HPLC-UV (see section 3.6.0) analysis of the methanol in the trap.

No cannabinoids were found on the HPLC-UV chromatogram which means that they were not dissolving in the methanol as it had been hoped. Furthermore, analyzing the inside of the vaporizer by rinsing it repeatedly with methanol showed that some of the cannabinoids had adhered to the inside of the glass enclosure, as a large CBN peak was shown on the HPLC-UV chromatogram. Why only the CBN peak was in abundance relative to the other major cannabinoids peaks typically seen is unclear.

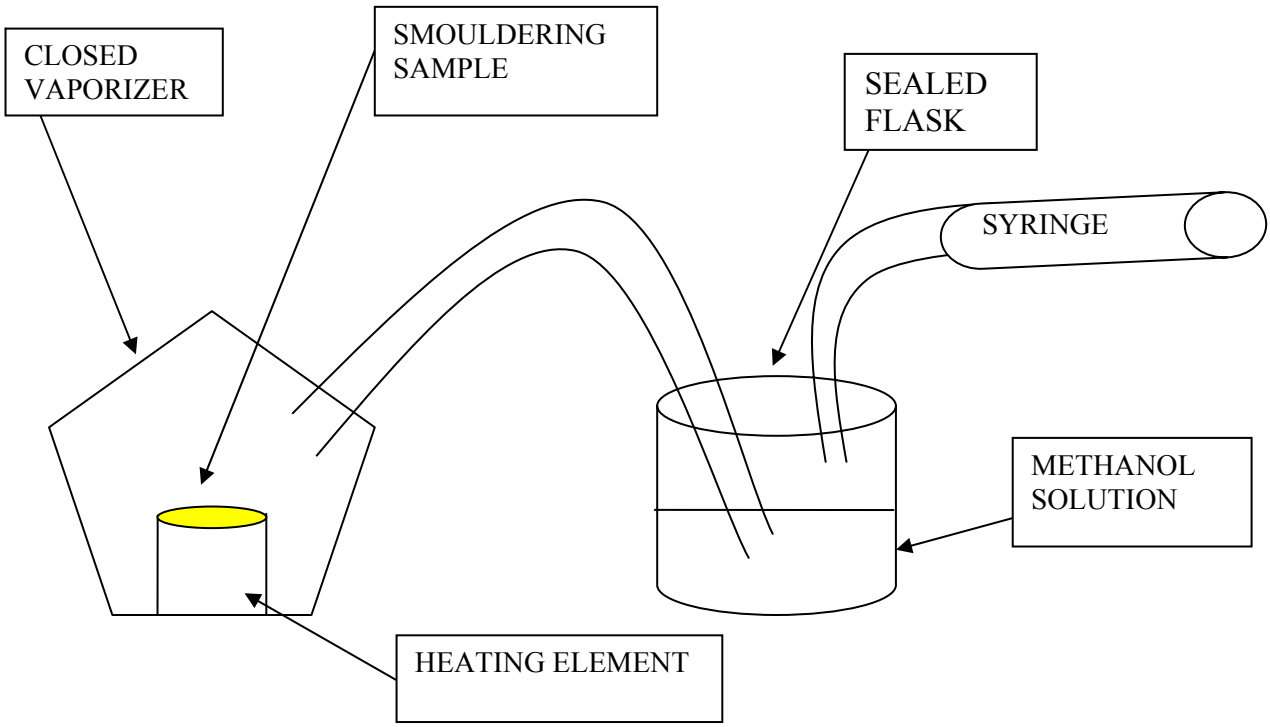


Figure 4.1 Smoking Machine #1

4.1.2.2 Smoking Machine #2

The second smoking machine that was employed involved the use of a gas sampling bulb. The gas sampling bulb is a circular glass vial 750 mL in capacity. At either end of it is two valves that can be opened and closed and are airtight when in the closed position. In the middle of the bulb is a one way syringe valve that can be used to sample the vapor inside the vesicle (see figure 4.2). The idea behind using the gas sampling vesicle was that by creating a vacuum inside of the bulb by using a vacuum pump, a negative pressure zone would develop. This negative pressure zone could then be used to draw a substance into the vesicle by opening one of the two valves at either end of the vesicle. The idea is that one could ignite a sample of cannabis plant and have the sample orifice connected to the gas sampling vesicle. Once the valve was open, the negative pressure would draw the combusted cannabis plant into the gas sampling vesicle which could then be rinsed with methanol and analyzed using HPLC-UV and HPLC-MS.

The biggest problem with this device was creating a suitable orifice and connecting it to the gas sampling vesicle so no sample would be lost to the external environment. The orifice created was milled with a drill press using brass fittings. A suitable fine mesh was then inserted into the orifice to hold the cannabis sample and a compression fitting was used to connect the brass pipe to the gas-sampling vesicle. The gas-sampling vesicle was then evacuated using the vacuum pump for ten minutes. At this point, the sample in the orifice was ignited, and the vacuum valve was opened

slightly to create a negative pressure zone and the smoke was drawn into the gas sampling vesicle.

Results indicated that it was impossible to evacuate the gas sampling vesicle enough to ensure a sustained vacuum. Without a sustained vacuum, the smoke escaped to atmosphere. Pressuring the vesicle for even 30 minutes still resulted in a vacuum that only lasting a few seconds. The problem is most likely the failure of the valve to control the vacuum at low levels. Hence, when the valve was opened, a large vacuum was created but only for a short period of time until atmospheric conditions were re-established.

Analyzing the inside of the gas sampling vesicle by repeated rinsing with methanol showed some interesting results. First of all, there was a large THC peak seen under HPLC-UV analysis with smaller peaks for CBD and CBN also showing up. The problem was too much of the combusted sample was escaping to atmosphere. This meant that although qualitatively cannabinoids were observed, quantitatively, their levels were far less then the plant's original THCA concentration assuming you had a 100% quantitative conversion to THC. This was expected as stated above: there was not enough sustained vacuum to move the combusted cannabinoids to inside the gas sampling vesicle before the entire cannabis sample was burned.

c

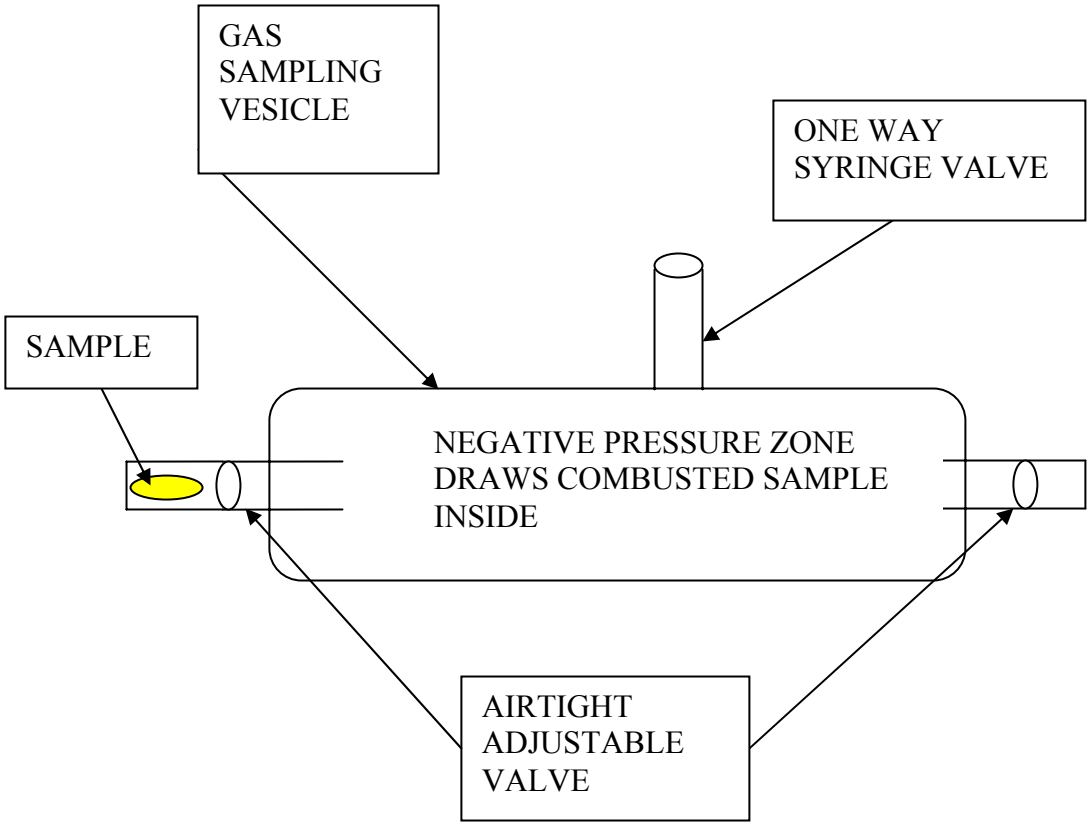


Figure 4.2 Smoking Machine #2

4.1.2.3 Smoking Machine #3

The third smoking machine that was devised provided a constant source of vacuum that could be user controllable for a specified length of time. Through the use of a rotary vacuum pump a constant vacuum could be created. So as to not simply lose all of the smoke and its contents to the vacuum pump oil, a "trap" was necessary between the combustion product and the vacuum pump. Consequently a medium was required that could reliably trap and contain all analytes with close to 100% recovery, which would ensure that the vacuum pump was not pulling everything past the media. Solid phase extraction cartridges offer characteristics similar to the columns that were being used to separate the cannabinoids in the HPLC-UV system and the HPLC-MS system. The first SPE cartridge used was a 3M Empore High Performance Extraction disk cartridge that had C18 standard density packing and was 7mm x 3mL in size. Another brass fitting was constructed that could have a fine mesh inserted into it to hold the sample. The brass sample container was then attached to an SPE cartridge that was then attached to a vacuum pump. The sample was then placed in the brass pipe and the vacuum was switched on (see figure 4.3). The cannabis was then ignited using a butane lighter and combusted to a nice white ash. The SPE cartridge was extracted using methanol and the extract was analyzed on HPLC-UV to determine the cannabinoids profile and recovery from the original sample. This setup worked very well as large amounts of THC were seen on the HPLC-UV as well as quantifiable amounts of CBD, CBN and CBC. It was decided at this point that this setup worked quite well and further investigations using this setup should be pursued.

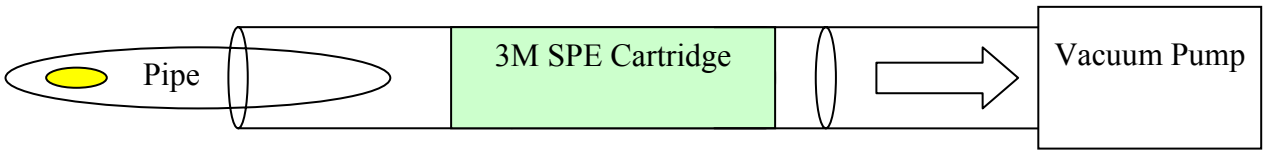


Figure 4.3 Smoking Machine #3

4.1.2.4 Smoking Machine #4

Smoking machine #3 worked very well as far as mimicking fairly accurately the actual process of smoking a cigarette. The problem with it was that some of the smoke was lost to atmosphere and also analyzing the sample holding area was a tedious process. Therefore, another idea was proposed that would follow the same basic principle of smoking machine #3 but also allow for the absolute measurement of all smoke produced and also an easier means to analyze the sample holding area. This would allow a more quantitative analysis of the cannabinoids since, in theory, it may be possible to not loose any sample after combustion. This machine worked by placing the sample to be ignited in the bottom of a test tube. A small piece of flexible hose was then attached to the test tube that had an SPE cartridge attached to it. The vacuum pump was then attached to the base of the SPE cartridge. The sample was heated using a Bunsen burner or butane lighter. Combustion could clearly be seen as smoke began to form inside the test tube. At this point, the vacuum was turned on and the smoke was pulled out of the test tube and deposited on the SPE cartridge. Once the sample showed only ash remaining, the vacuum was turned off. The SPE cartridge was then extracted with 1 mL of methanol on the vacuum extraction manifold. At the same time, the test tube had 1 mL of methanol added to it and was capped and then mixed on a vibrax shaker at speed setting 1000 for 10 minutes. The sample was filtered and placed in storage tube so it could be analyzed by HPLC-UV (see method 3.6.0).

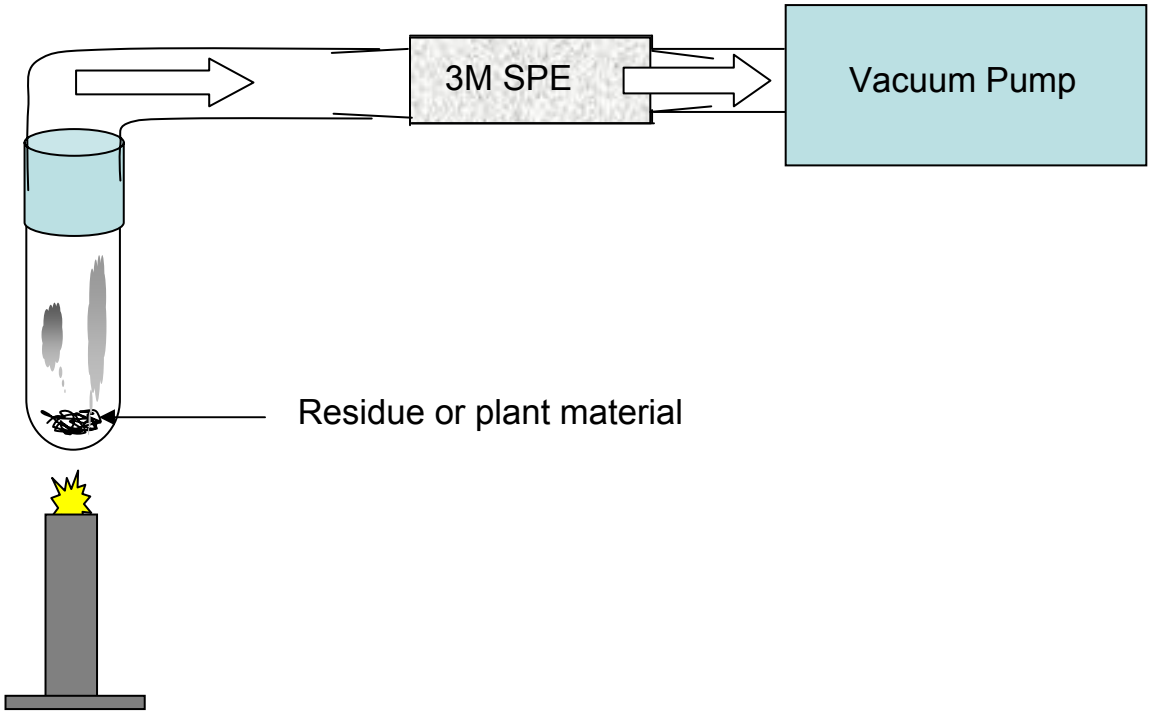


Figure 4.4 Smoking Machine #4

4.1.2.5 Other Devices Used in the Analytical Process to Determine the Combustion Profile of Cannabis Sativa Plant

To further verify the effect of heat on cannabis plant tissue, a number of experiments were carried out looking at the effect varying amounts of heat and various temperatures had on the cannabinoid profile of marihuana. In order to do this, a convection oven from an HP 5790 gas chromatograph capable of temperature programming was used. A sample of cannabis plant with a known concentration of THCA was placed inside a glass test tube and then capped with a heat stable screw cap. The experiments were then performed at varying temperatures from ambient to 250°C and time duration of zero to thirty minutes The test tubes were then removed from the oven and allowed to cool. After cooling, 3 mL of methanol was added to each test tube and mixed on a vibrax shaker speed setting 1000 for ten minutes. The methanol extract was then filtered and placed inside a suitable vial that could then be analyzed under HPLC-UV and/or HPLC-MS/MS.

4.2 Intra And Inter – Assay Precision And Accuracy For THC, CBD, CBN And THCA

The HPLC-UV method used to quantify and analyze qualitatively the cannabinoids of interest was validated previously (Dautbegovic et al, 2003). However, it was felt important to re-validate the results of this method since it employs a much smaller sample size (5mg versus 100mg) and was to be used by a different analyst.

A re-validation of the original method was undertaken to demonstrate inter-assay variability. The inter-assay % CV and observed % difference from nominal could be

used to attest to the precision and accuracy of the method since it demonstrates assay performance on different days by an alternate analyst. Table 4.1 shows the %CV was less than 4.5 % across the range of concentrations 12.5 μg/mL to 200 μg/mL for CBD. Similarly for CBN (Table 4.2) the inter-assay %CV was less than 5.47 % across the calibration range. THC (Table 4.3) demonstrated 6.95 % or less variation between days of analysis across the calibration range. THCA %CV inter-assay variation was no greater then 4.49% across the calibration range (Table 4.4).

The quality control samples included duplicates at the low concentration (37.5 μg/mL), mid-point concentration (75 μg/mL) and at a high concentration (150 μg/mL). The quality control (QC) samples analyzed with these calibrators demonstrated that 37/38 of QC's at all concentrations had a bias (% difference from actual concentration) of less than ±15 % for CBD (table 4.5), CBN (table 4.6), THC (table 4.7) and THCA (table 4.8). The only problematic QC was one of the two mid point concentrations on day 1. It consistently gave a value of approximately 16% difference for CBD, CBN, THC and THCA. Furthermore the %CV of the QC's across all days for each analyte at all concentration levels was always less then 5%.

Table 4.1 Revalidation of HPLC-UV method for CBD Standard

REVALIDATION OF HPLC-UV METHOD										
CBD OBSERVED CONCENTRATION FOR THE FOLLOWING STANDARDS µg/mL										
DAY	**12.5**	**% diff**	**25**	**% diff**	**50**	**% diff**	**100**	**% diff**	**200**	**% diff**
1	*	*	*	*	*	*	*	*	*	*
	12.73	1.81	24.90	-0.38	50.53	1.05	98.72	-1.28	200.25	0.13
2	11.67	-6.63	24.37	-2.52	51.07	2.15	105.34	5.34	204.18	2.09
	11.31	-9.51	24.11	-3.55	49.58	-0.84	100.12	0.12	193.24	-3.38
3	12.29	-1.70	26.78	7.12	52.07	4.14	106.61	6.61	*****	*
	12.57	0.54	23.94	-4.24	46.94	-6.13	93.52	-6.48	190.04	-4.98
4	13.04	4.33	24.23	-3.09	48.90	-2.21	100.83	0.83	196.39	-1.80
	13.15	5.20	24.73	-1.10	49.48	-1.04	102.14	2.14	203.61	1.80
5	11.85	-5.23	24.70	-1.22	49.55	-0.91	100.21	0.21	196.78	-1.61
	11.99	-4.10	25.25	0.98	50.93	1.85	101.58	1.58	202.51	1.26
6	11.85	-5.23	24.70	-1.22	49.55	-0.91	100.21	0.21	196.78	-1.61
	11.99	-4.10	25.25	0.98	50.93	1.85	101.58	1.58	202.51	1.26
7	11.85	-5.23	24.70	-1.22	49.55	-0.91	100.21	0.21	196.78	-1.61
	11.99	-4.10	25.25	0.98	50.93	1.85	101.58	1.58	202.51	1.26
MEAN	12.17		24.84		50.00		100.97		198.80	
SD	0.55		0.72		1.29		3.11		4.48	
%CV	4.50		2.91		2.58		3.08		2.26	

***** All data was analyzed in replicates of two for each concentration over a 7 day period.
****** Datum for day 1 was discarded due to a problem with the autosampler injecting the wrong volume of sample. Datum for day 3 at 200 µg/mL was discarded due to contamination.

Table 4.2 Revalidation of HPLC-UV method for CBN Standard

REVALIDATION OF HPLC-UV METHOD										
CBN OBSERVED CONCENTRATION FOR THE FOLLOWING STANDARDS µg/mL										
RUN #	**12.5**	**% diff**	**25**	**% diff**	**50**	**% diff**	**100**	**% diff**	**200**	**% diff**
1	*	*	*	*	*	*	*	*	*	*
	10.78	-13.79	23.18	-7.28	48.65	-2.71	98.12	-1.88	198.34	-0.83
2	10.69	-14.52	24.35	-2.61	51.56	3.11	106.06	6.06	203.86	1.93
	10.91	-12.71	23.56	-5.76	50.28	0.55	101.36	1.36	192.65	-3.67
3	12.35	-1.18	26.56	6.24	51.97	3.95	106.58	6.58	*****	*
	12.39	-0.88	24.44	-2.23	47.08	-5.84	93.82	-6.18	187.52	-6.24
4	12.43	-0.56	23.93	-4.28	49.10	-1.80	101.50	1.50	196.18	-1.91
	12.39	-0.92	24.52	-1.93	49.97	-0.06	103.24	3.24	202.27	1.14
5	11.30	-9.62	24.44	-2.23	50.00	-0.01	101.31	1.31	196.34	-1.83
	11.46	-8.32	25.08	0.32	50.73	1.47	102.38	2.38	201.55	0.78
6	11.30	-9.62	24.44	-2.23	50.00	-0.01	101.31	1.31	196.34	-1.83
	11.46	-8.32	25.08	0.32	50.73	1.47	102.38	2.38	201.55	0.78
7	11.30	-9.62	24.44	-2.23	50.00	-0.01	101.31	1.31	196.34	-1.83
	11.46	-8.32	25.08	0.32	50.73	1.47	102.38	2.38	201.55	0.78
MEAN	11.55		24.55		50.06		101.67		197.87	
SD	0.63		0.83		1.26		3.19		4.68	
%CV	5.47		3.37		2.52		3.14		2.36	

***** All data was analyzed in replicates of two for each concentration over a 7 day period.
****** Datum for day 1 was discarded due to a problem with the autosampler injecting the wrong volume of sample. Datum for day 3 at 200 µg/mL was discarded due to contamination.

Table 4.3 Revalidation of HPLC-UV method for THC Standard

REVALIDATION OF HPLC-UV METHOD										
THC OBSERVED CONCENTRATION FOR THE FOLLOWING STANDARDS µg/mL										
RUN #	**12.5**	**% diff**	**25**	**% diff**	**50**	**% diff**	**100**	**% diff**	**200**	**% diff**
1	*	*	*	*	*	*	*	*	*	*
	13.66	9.28	25.16	0.62	49.68	-0.64	97.59	-2.41	200.70	0.35
2	10.86	-13.09	22.40	-10.40	46.39	-7.23	95.24	-4.76	186.90	-6.55
	11.13	-10.97	21.94	-12.23	45.01	-9.98	91.12	-8.88	176.67	-11.67
3	12.76	2.09	26.34	5.36	51.42	2.83	107.31	7.31	*****	*
	13.10	4.80	24.03	-3.90	46.45	-7.11	93.46	-6.54	193.31	-3.35
4	13.25	6.02	24.40	-2.40	48.09	-3.81	99.48	-0.52	196.88	-1.56
	14.06	12.49	25.19	0.75	49.66	-0.69	101.22	1.22	204.28	2.14
5	12.59	0.73	24.82	-0.71	48.82	-2.36	98.82	-1.18	196.53	-1.74
	13.02	4.14	25.62	2.47	50.44	0.88	100.76	0.76	204.18	2.09
6	12.59	0.73	24.82	-0.71	48.82	-2.36	98.82	-1.18	196.53	-1.74
	13.02	4.14	25.62	2.47	50.44	0.88	100.76	0.76	204.18	2.09
7	12.59	0.73	24.82	-0.71	48.82	-2.36	98.82	-1.18	196.53	-1.74
	13.02	4.14	25.62	2.47	50.44	0.88	100.76	0.76	204.18	2.09
MEAN	12.74		24.67		48.81		98.78		196.74	
SD	0.88		1.26		1.88		4.00		8.26	
%CV	6.95		5.12		3.86		4.05		4.20	

***** All data was analyzed in replicates of two for each concentration over a 7 day period.
****** Datum for day 1 was discarded due to a problem with the autosampler injecting the wrong volume of sample. Datum for day 3 at 200 µg/mL was discarded due to contamination.

Table 4.4 Revalidation of HPLC-UV method for THCA Standard

REVALIDATION OF HPLC-UV METHOD										
THCA OBSERVED CONCENTRATION FOR THE FOLLOWING STANDARDS µg/mL										
RUN #	**12.5**	**% diff**	**25**	**% diff**	**50**	**% diff**	**100**	**% diff**	**200**	**% diff**
1	*	*	*	*	*	*	*	*	*	*
	12.53	0.23	25.06	0.25	50.61	1.21	99.05	-0.95	200.45	0.22
2	11.34	-9.28	25.16	0.62	50.74	1.48	104.72	4.72	206.10	3.05
	11.30	-9.64	24.38	-2.48	50.09	0.18	99.50	-0.50	192.02	-3.99
3	12.32	-1.44	26.89	7.56	51.34	2.69	107.38	7.38	*****	*
	12.64	1.14	24.01	-3.98	47.11	-5.78	92.84	-7.16	190.53	-4.74
4	12.78	2.23	23.83	-4.66	49.04	-1.92	100.89	0.89	197.14	-1.43
	13.29	6.31	24.52	-1.93	49.19	-1.62	102.55	2.55	201.60	0.80
5	12.60	0.83	24.81	-0.75	48.92	-2.16	98.51	-1.49	195.99	-2.00
	12.75	2.00	25.94	3.75	50.42	0.84	101.04	1.04	204.89	2.45
6	12.60	0.83	24.81	-0.75	48.92	-2.16	98.51	-1.49	195.99	-2.00
	12.75	2.00	25.94	3.75	50.42	0.84	101.04	1.04	204.89	2.45
7	12.60	0.83	24.81	-0.75	48.92	-2.16	98.51	-1.49	195.99	-2.00
	12.75	2.00	25.94	3.75	50.42	0.84	101.04	1.04	204.89	2.45
MEAN	12.48		25.08		49.70		100.43		199.21	
SD	0.56		0.88		1.14		3.46		5.34	
%CV	4.49		3.49		2.29		3.45		2.68	

***** All data was analyzed in replicates of two for each concentration over a 7 day period.
****** Datum for day 1 was discarded due to a problem with the autosampler injecting the wrong volume of sample. Datum for day 3 at 200 µg/mL was discarded due to contamination.

Table 4.5 Revalidation of HPLC-UV method for CBD Quality Controls

REVALIDATION OF HPLC-UV METHOD						
CBD OBSERVED CONCENTRATION FOR THE FOLLOWING QUALITY CONTROLS						
DAY	**QC1 37.5 µg/mL**	**% diff**	**QC2 75 µg/mL**	**% diff**	**150 µg/mL**	**% diff**
1	37.78	0.74	78.14	4.19	154.12	2.75
	42.78	14.07	87.74	16.99	168.59	12.39
2	*		82.46	9.95	160.90	7.27
	*		82.48	9.97	160.41	6.94
3	*		84.06	12.08	148.98	-0.68
	*		85.14	13.52	147.24	-1.84
4	38.51	2.68	78.95	5.26	160.56	7.04
	38.99	3.98	80.56	7.41	159.84	6.56
5	39.06	4.16	79.32	5.75	155.93	3.95
	38.25	2.00	77.87	3.83	153.67	2.45
6	39.06	4.16	79.32	5.75	155.93	3.95
	38.25	2.00	77.87	3.83	153.67	2.45
7	39.06	4.16	79.32	5.75	155.93	3.95
	38.25	2.00	77.87	3.83	153.67	2.45
MEAN	39.13		81.00		156.56	
SD	1.42		3.14		5.59	
%CV	3.62		3.88		3.57	

***** All data was analyzed in replicates of two for each concentration over a 7 day period.
** Day 2 and Day 3 at the 37.5 µg/mL quality control amount were discarded due to a problem with the autosampler injecting the wrong volume of sample.

Table 4.6 Revalidation of HPLC-UV method for CBN Quality Controls

REVALIDATION OF HPLC-UV METHOD						
CBN OBSERVED CONCENTRATION FOR THE FOLLOWING QUALITY CONTROLS						
DAY	**QC1 37.5 µg/mL**	**% diff**	**QC2 75 µg/mL**	**% diff**	**150 µg/mL**	**% diff**
1	36.26	-3.31	76.50	2.00	152.69	1.80
	41.72	11.26	87.13	16.17	167.59	11.73
2	*		83.13	10.84	161.52	7.68
	*		82.54	10.06	161.32	7.55
3	*		84.45	12.60	147.33	-1.78
	*		84.98	13.31	146.66	-2.23
4	38.76	3.35	80.02	6.70	160.93	7.28
	39.28	4.75	81.64	8.85	159.98	6.65
5	38.96	3.88	80.29	7.06	156.41	4.27
	38.39	2.38	78.80	5.06	154.67	3.12
6	38.96	3.88	80.29	7.06	156.41	4.27
	38.39	2.38	78.80	5.06	154.67	3.12
7	38.96	3.88	80.29	7.06	156.41	4.27
	38.39	2.38	78.80	5.06	154.67	3.12
MEAN	39.09		81.63		156.81	
SD	1.04		2.65		5.72	
%CV	2.65		3.25		3.65	

***** All data was analyzed in replicates of two for each concentration over a 7 day period.
** Day 2 and Day 3 at the 37.5 µg/mL quality control amount were discarded due to a problem with the autosampler injecting the wrong volume of sample.

Table 4.7 Revalidation of HPLC-UV method for THC Quality Controls

REVALIDATION OF HPLC-UV METHOD						
THC OBSERVED CONCENTRATION FOR THE FOLLOWING QUALITY CONTROLS						
DAY	**QC1 37.5 µg/mL**	**% diff**	**QC2 75 µg/mL**	**% diff**	**150 µg/mL**	**% diff**
1	37.73	0.63	77.12	2.83	153.25	2.17
	42.31	12.83	87.06	16.09	168.21	12.14
2	*		74.86	-0.19	146.67	-2.22
	*		74.35	-0.86	146.39	-2.41
3	*		83.52	11.37	149.25	-0.50
	*		84.92	13.22	149.28	-0.48
4	38.33	2.23	77.62	3.50	158.92	5.95
	38.66	3.09	79.03	5.38	159.33	6.22
5	38.51	2.69	78.09	4.12	154.55	3.03
	37.78	0.74	76.59	2.12	152.91	1.94
6	38.51	2.69	78.09	4.12	154.55	3.03
	37.78	0.74	76.59	2.12	152.91	1.94
7	38.51	2.69	78.09	4.12	154.55	3.03
	37.78	0.74	76.59	2.12	152.91	1.94
MEAN	38.68		78.88		153.88	
SD	1.41		3.88		5.88	
%CV	3.64		4.92		3.82	

***** All data was analyzed in replicates of two for each concentration over a 7 day period.
** Day 2 and Day 3 at the 37.5 µg/mL quality control amount were discarded due to a problem with the autosampler injecting the wrong volume of sample.

Table 4.8 Revalidation of HPLC-UV method for THCA Quality Controls

REVALIDATION OF HPLC-UV METHOD						
THCA OBSERVED CONCENTRATION FOR THE FOLLOWING QUALITY CONTROLS						
DAY	**QC1 37.5 µg/mL**	**% diff**	**QC2 75 µg/mL**	**% diff**	**150 µg/mL**	**% diff**
1	36.86	-1.71	76.87	2.49	153.78	2.52
	42.19	12.51	88.74	18.32	168.91	12.61
2	*		83.62	11.49	161.92	7.95
	*		82.64	10.19	159.20	6.13
3	*		83.84	11.79	148.58	-0.94
	*		84.17	12.22	142.24	-5.18
4	38.35	2.26	79.31	5.75	160.43	6.96
	39.40	5.07	81.24	8.31	155.13	3.42
5	39.40	5.06	78.96	5.28	154.41	2.94
	37.91	1.10	76.58	2.10	152.22	1.48
6	39.40	5.06	78.96	5.28	154.41	2.94
	37.91	1.10	76.58	2.10	152.22	1.48
7	39.40	5.06	78.96	5.28	154.41	2.94
	37.91	1.10	76.58	2.10	152.22	1.48
MEAN	39.10		80.78		155.10	
SD	1.36		3.66		6.56	
%CV	3.47		4.53		4.23	

***** All data was analyzed in replicates of two for each concentration over a 7 day period.
** Day 2 and Day 3 at the 37.5 µg/mL quality control amount were discarded due to a problem with the autosampler injecting the wrong volume of sample.

4.3 Solid Phase Extraction Recovery

The first experiment carried out was to determine the % recovery of the 3M C18 SPE cartridge using liquid standards. This was accomplished by taking standards of CBD, CBN, THC and THCA and running them through the cartridge. The cartridge was

mounted on a Waters vacuum filtration extraction system capable of extracting 20 samples at a time. A sample size of 1 mL was injected onto the cartridge with a concentration of 100 µg/mL for each standard. After the samples had stopped flowing through the cartridge, the vacuum system was turned on and stayed on until a pressure – 20 mm Hg was obtained. Clean test tubes were placed underneath the cartridges and the methanol was collected and the resulting eluent was analyzed. Each standard was reconstituted in 20% methanol / water and then extracted using 100% methanol. The reason for this was cannabinoids dissolve so well in methanol that it was hard to get them to adhere to the column if the solvent contained too much methanol. Less then 20% methanol resulted in precipitation and clogging up the SPE cartridge. Each standard was analyzed in replicates of 5. Table 4.9 shows THC standard at a concentration of 100 µg/mL. It can be seen that the average concentration recovered was 88.98 µg/mL with a %CV of 3.09. Table 4.10 shows CBD standard at a concentration of 100 µg/mL. It can be seen that the average concentration recovered was 90.18 µg/mL with a %CV of 2.56. Table 4.11 shows CBN standard at a concentration of 100 µg/mL. It can be seen that the average concentration recovered was 88.06 µg/mL with a %CV of 2.46. Lastly, Table 4.12 shows THCA standard at a concentration of 100 µg/mL. It can be seen that the average concentration recovered was 88.58 µg/mL with a %CV of 2.44.

Table 4.9 Recovery of THC using 3M C18 SPE cartridges, concentration of THC was 100µg/mL

SAMPLE	**CONCENTRATION THC RECOVERED**
1	89.3
2	88.8
3	89.5
4	84.8
5	92.5
AVERAGE	88.98
SD	2.75
%CV	3.09

Table 4.10 Recovery of CBD using 3M C18 SPE cartridges, concentration of CBD was 100 µg/mL

SAMPLE	**CONCENTRATION CBD RECOVERED**
1	92.4
2	92.8
3	87.5
4	88.9
5	89.3
AVERAGE	90.18
SD	2.31
%CV	2.56

Table 4.11 Recovery of CBN using 3M C18 SPE cartridges, concentration of CBN was 100 µg/mL

SAMPLE	**CONCENTRATION CBN RECOVERED**
1	89.4
2	84.8
3	88.5
4	87.2
5	90.4
AVERAGE	88.06
SD	2.16
%CV	2.46

Table 4.12 Recovery of THCA using 3M C18 SPE cartridges, concentration of THCA was 100 µg/mL

SAMPLE	**CONCENTRATION THCA RECOVERED**
1	88.8
2	89.3
3	85.6
4	91.5
5	87.7
AVERAGE	88.58
SD	2.16
%CV	2.44

4.4 Cannabis Sativa Cannabinoids HPLC-MS-MS Profiles

In order to evaluate the cannibinoid profile of a typical marihuana plant, the analytical procedure as stated in section 3.7.0 was carried out on standards of CBD, CBN, THC, THCA and CBC. This was necessary to determine the mass spectral characteristics of each cannabinoid under MS and MS/MS conditions. The reason for doing this was to create a known ion chromatogram library that could be used as a reference later on when looking at the combustion products of cannabis.

Figure 4.5 is the full scanning ion chromatogram for CBD. It can clearly be seen that the base peak and the molecular ion is seen at a mass of 315. The product ion scan of the 315 mass is shown in figure 4.6. It shows a large peak at 123 and a smaller peak at 193 mass.

Figure 4.7 is the full scanning ion chromatogram for CBN. It can be seen that the base peak and molecular ion is seen at a mass of 311. The product ion scan of the 311 mass is shown in figure 4.8. It shows a predominant peak at mass 223.

Figure 4.9 is the full scanning ion chromatogram for THC. It can be seen that the base peak and molecular ion is seen at a mass of 315. It is notable that the molecular weight for CBD and THC is the same. The only difference between the molecules is a ring opening on CDB (see figure 1.4). The product ion scan of the 315 mass is shown in figure 4.10. It shows a base peak at mass 193 with smaller peaks at 123, 135 and 259.

Figure 4.11 is the full scanning ion chromatogram for THCA. It can be seen that the base peak is seen at a mass of 391 and the molecular ion is seen at a mass of 359. Under electrospray positive mode, it is difficult to add a $[H^+]$ ion to the THCA molecule as under low pH conditions, the acidic site on the THCA molecule will already be protonated from the acidic mobile phase. As a result of this, the base peak differs from

the molecular ion under electrospray positive conditions. The product ion scan of the 359 mass is shown in figure 4.12. It shows a predominant peak at mass 219 and a smaller peak at mass 341.

Figure 4.13 is the full scanning ion chromatogram for CBC. It can be seen that the base peak and molecular ion is seen at a mass of 315. The product ion scan of the 315 mass is shown in figure 4.14. It shows a predominant peak at mass 193.

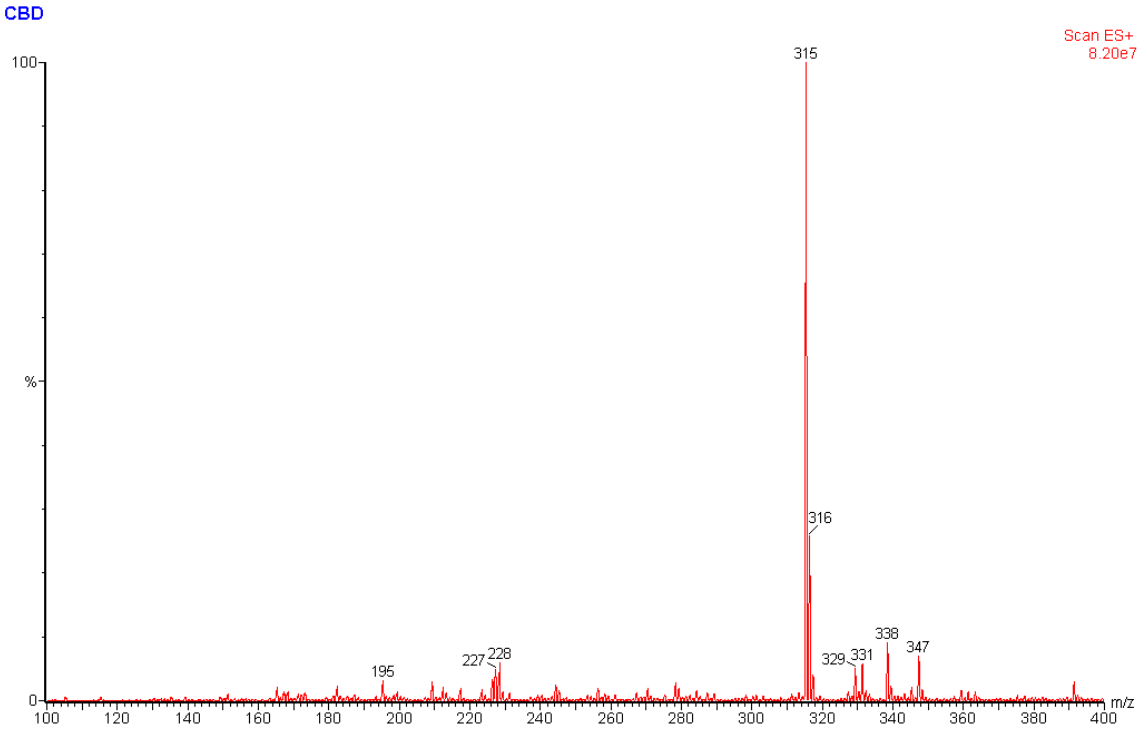


Figure 4.5 CBD full scanning Ion Chromatogram from Micromass Ultima Triple Quadrapole Mass Spectrometer using electrospray positive ion mode and using analytical procedure stated in section 3.7.0

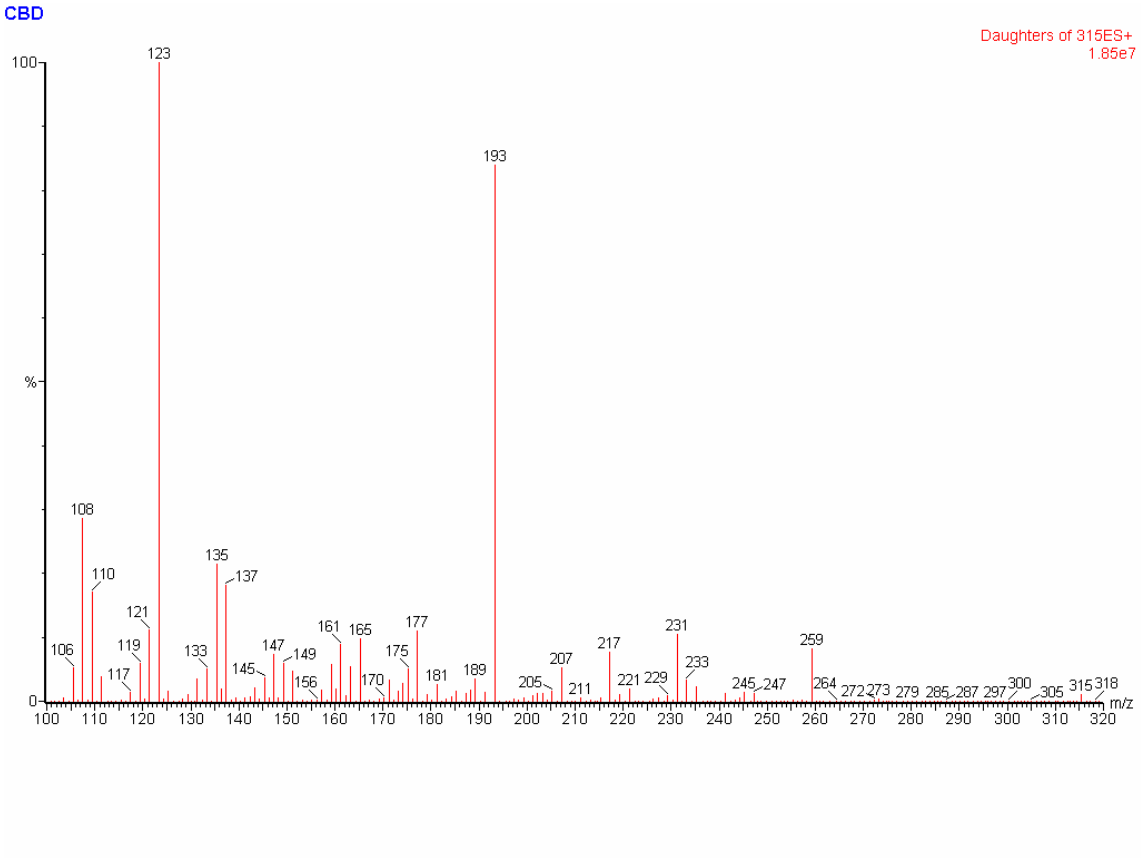


Figure 4.6 CBD product ion scan from Micromass Ultima Triple Quadrapole Mass Spectrometer using electrospray positive ion mode and using analytical procedure stated in section 3.7.0

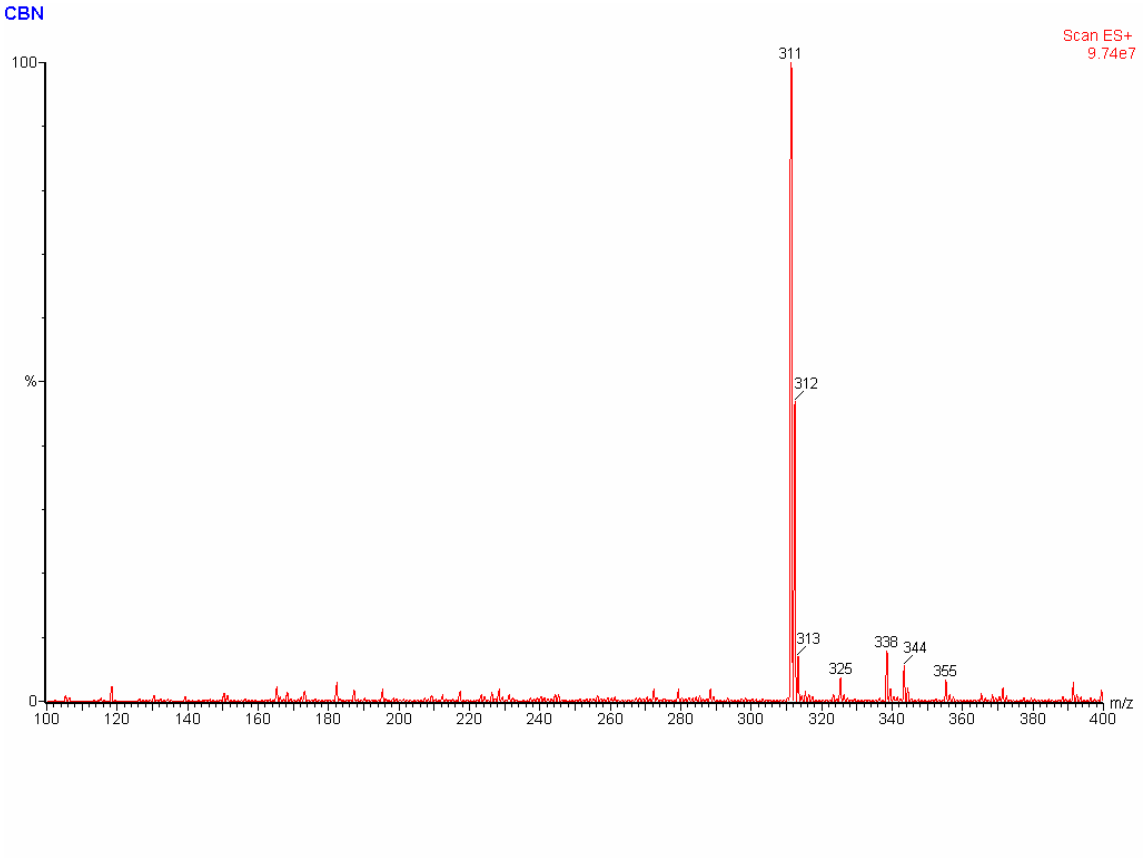


Figure 4.7 CBN full scanning ion chromatogram from Micromass Ultima Triple Quadrapole Mass Spectrometer using electrospray positive ion mode with and using analytical procedure stated in section 3.7.0

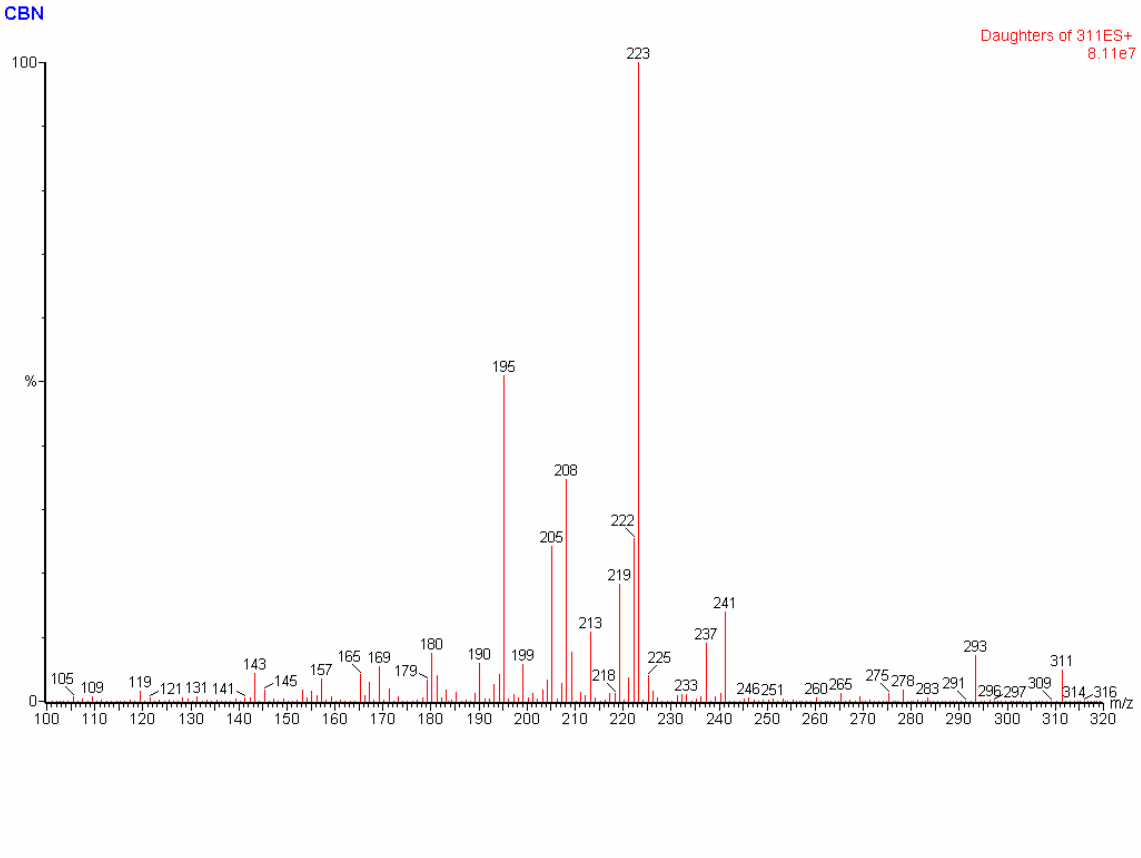


Figure 4.8 CBN product ion chromatogram from Micromass Ultima Triple Quadrapole Mass Spectrometer using electrospray positive ion mode and using analytical procedure stated in section 3.7.0

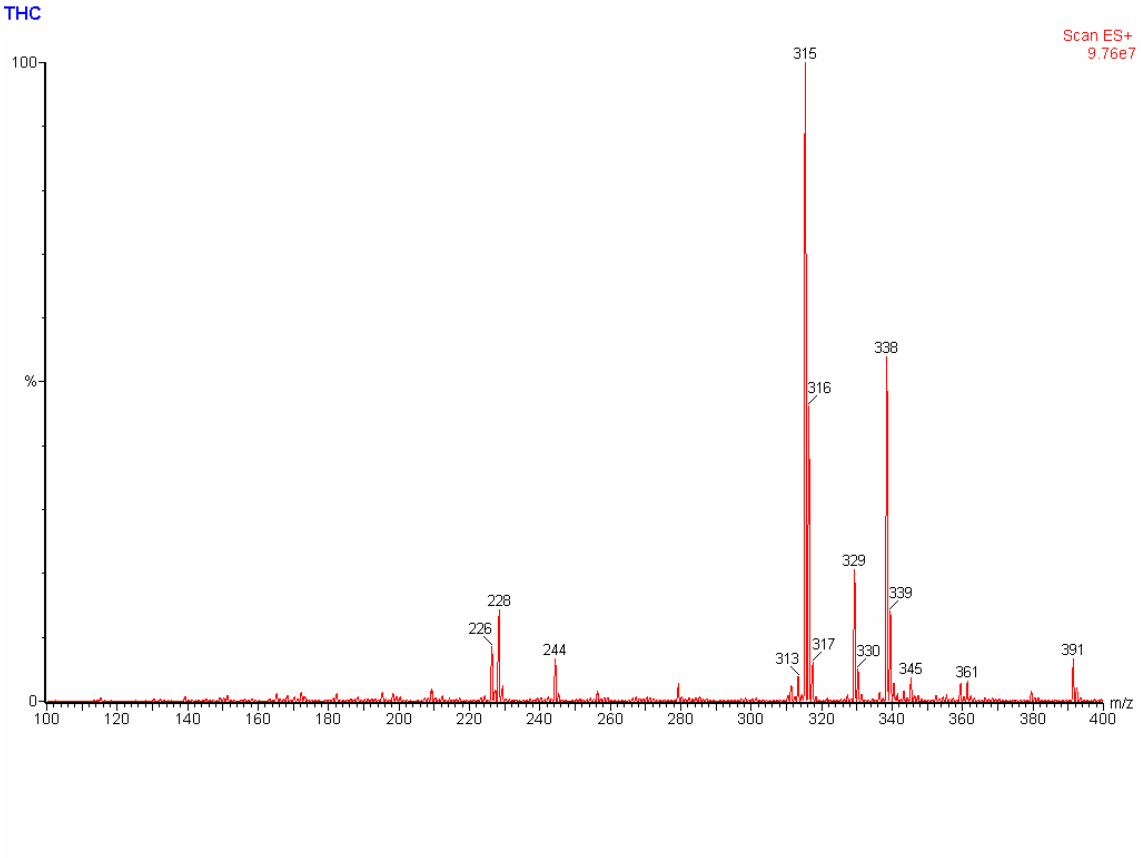


Figure 4.9 THC full scanning ion chromatogram from Micromass Ultima Triple Quadrapole Mass Spectrometer using electrospray positive ion mode and using analytical procedure stated in section 3.7.0

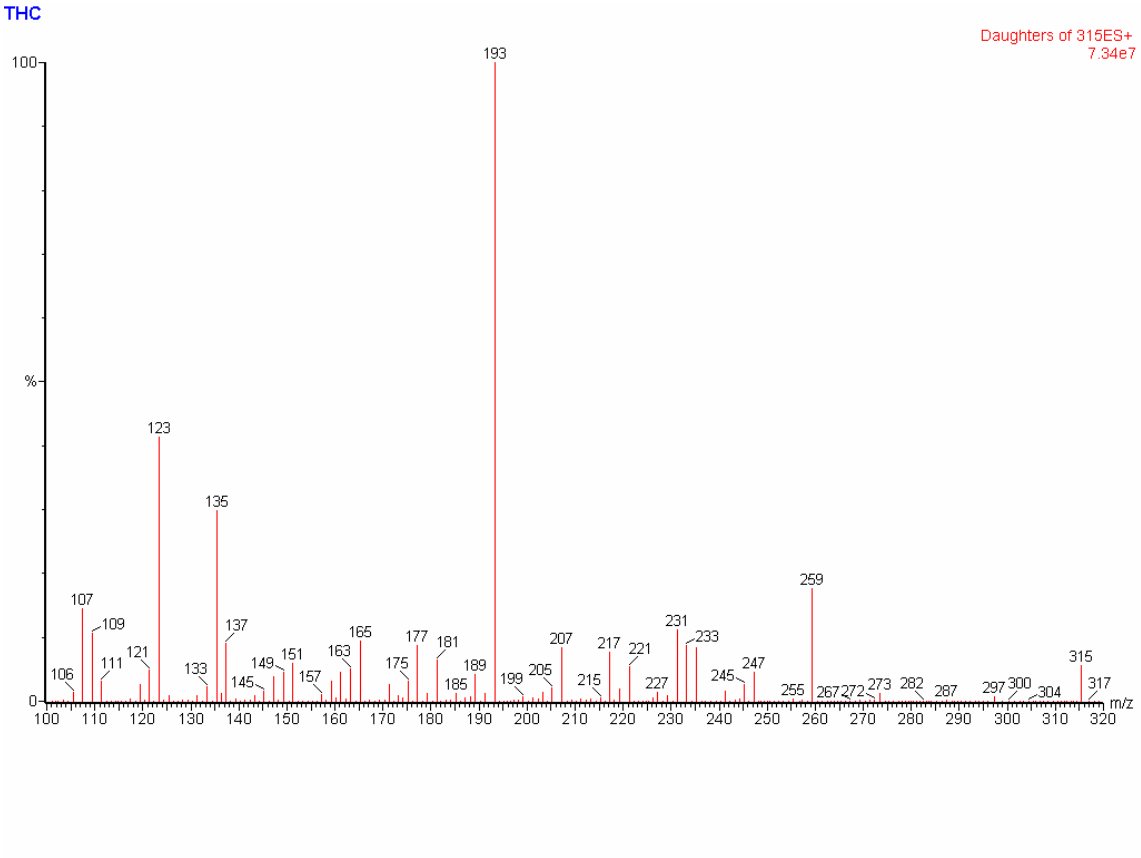


Figure 4.10 THC product Ion scan from Micromass Ultima Triple Quadrapole Mass Spectrometer using electrospray positive ion mode and using analytical procedure stated in section 3.7.0

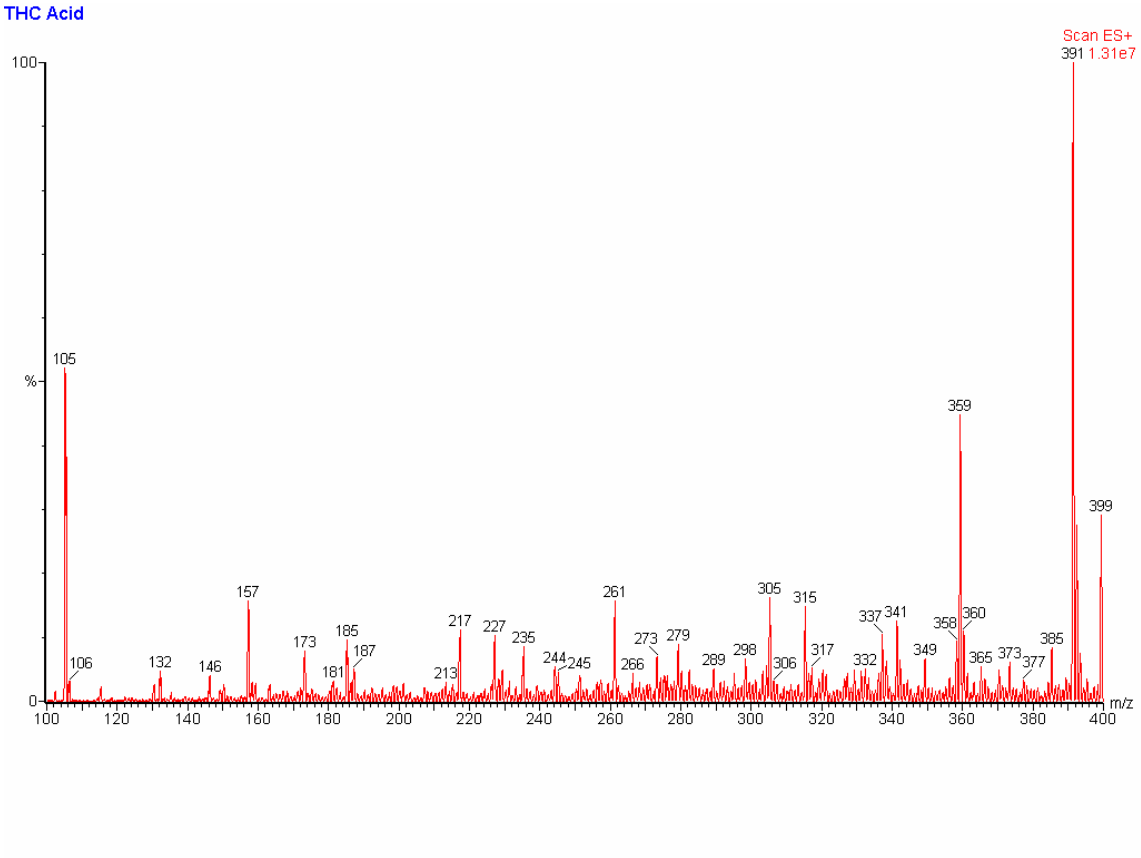


Figure 4.11 THCA full scanning ion chromatogram from Micromass Ultima Triple Quadrapole Mass Spectrometer using electrospray positive ion mode and using analytical procedure stated in section 3.7.0

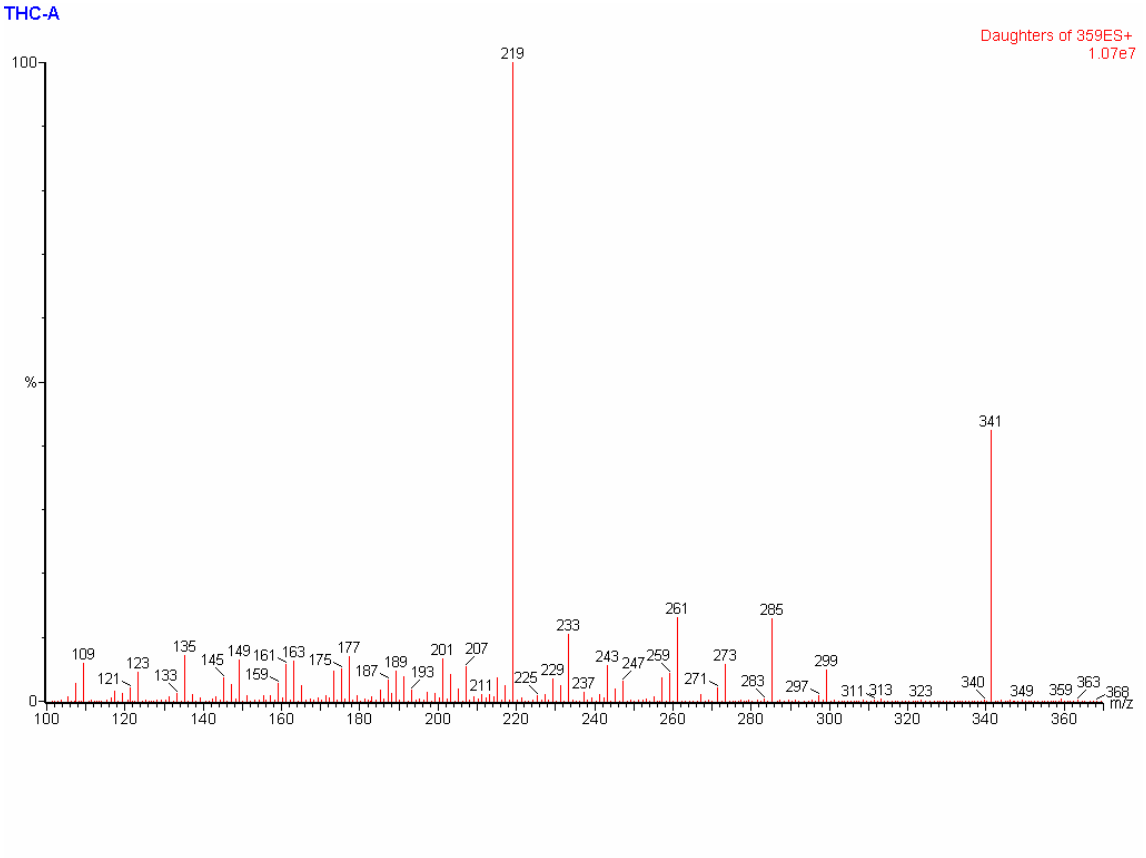


Figure 4.12 THCA product ion scan from Micromass Ultima Triple Quadrapole Mass Spectrometer using electrospray positive ion mode and using analytical procedure stated in section 3.7.0

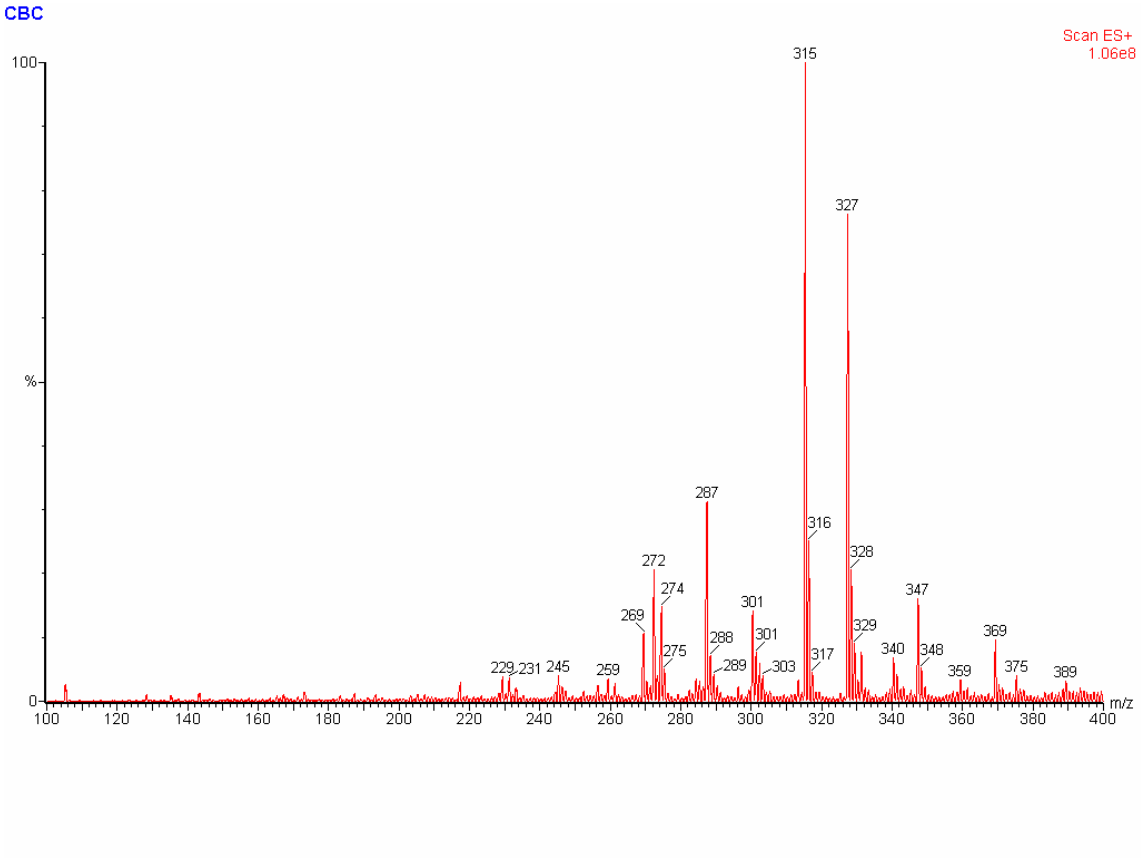


Figure 4.13 CBC full scan ion chromatogram from Micromass Ultima Triple Quadrapole Mass Spectrometer using electrospray positive ion mode and using analytical procedure stated in section 3.7.0

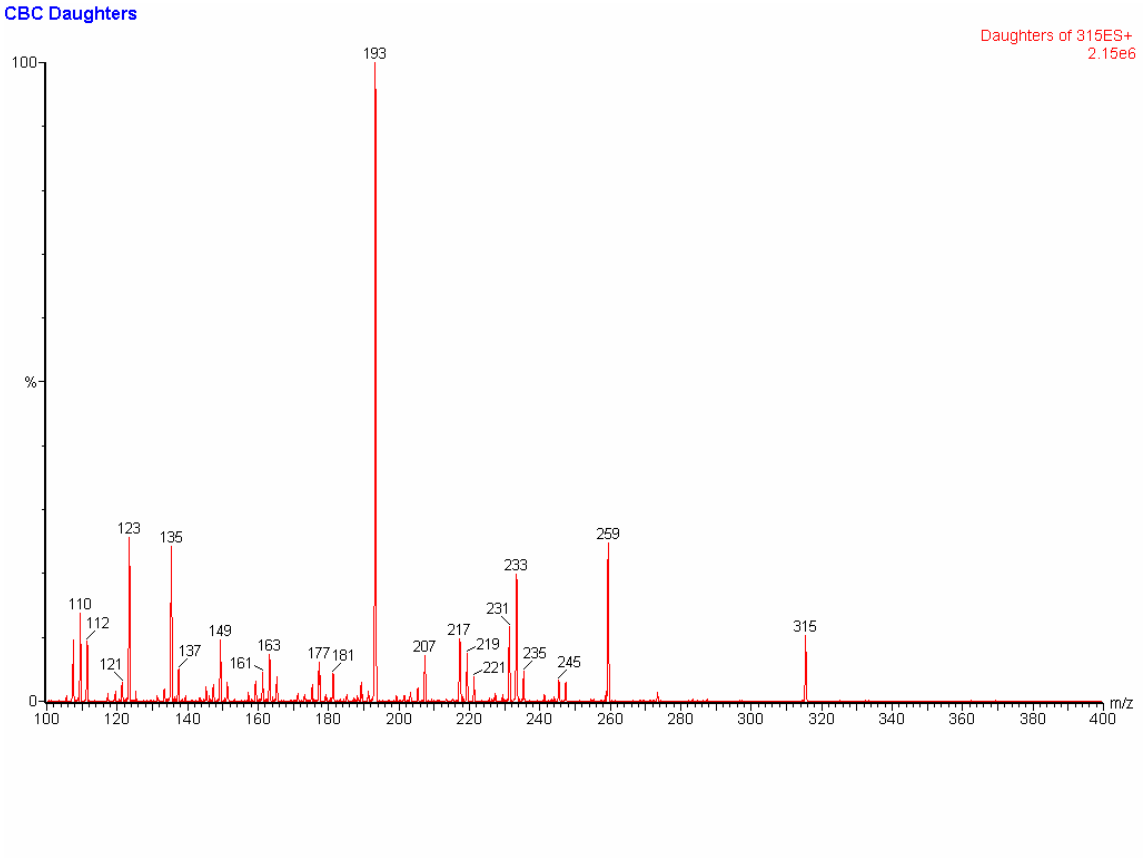


Figure 4.14 CBC product ion scan from a Micromass Ultima Triple Quadrapole Mass Spectrometer using electrospray positive ion mode and using analytical procedure stated in section 3.7.0

4.4.1 Cannabis Sativa Cannabinoids HPLC-UV Spectrophotometer Profile

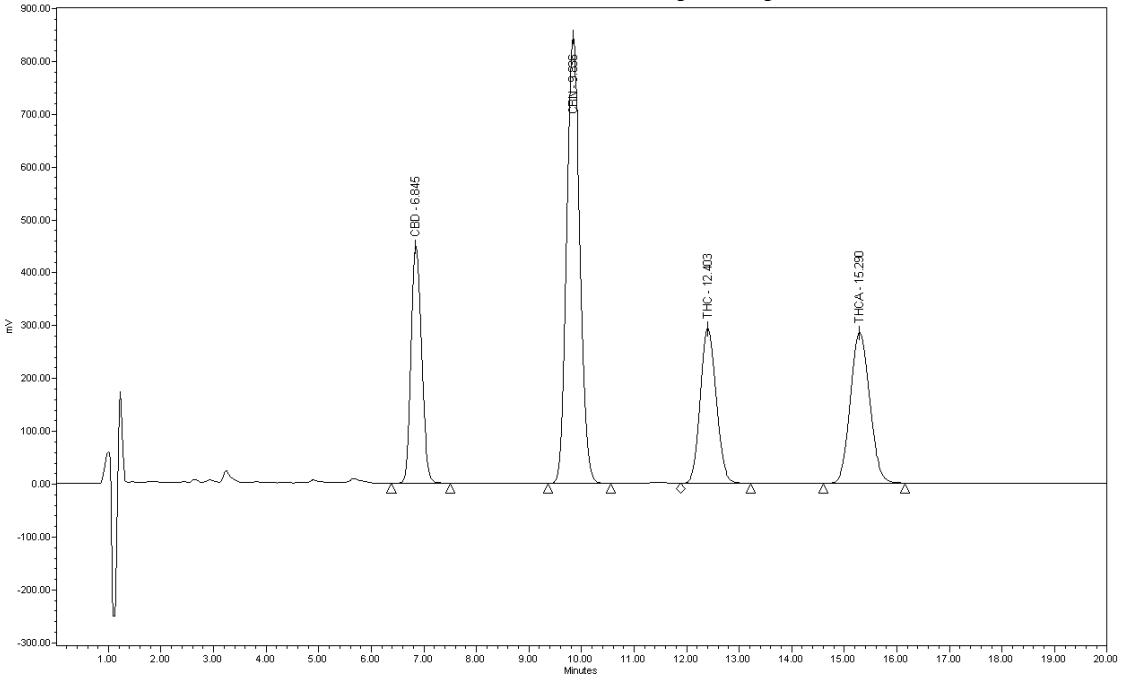


Figure 4.15 HPLC-UV chromatogram of CBD, CBN, THC and THCA at 200 µg/mL

The above chromatogram is a typical chromatographic profile of the 4 major compounds found in a cannabis sativa plant. The retention time shown and peak areas are typical for each compound at 200 µg/mL in the HPLC-UV analytical system used here (see section 3.6.0).

To determine the capabilities of the extraction system, a number of experiments were performed. Each experiment was designed to validate the method in terms of reproducibility and efficiency of the extraction system.

4.5 Stability of Cannabinoids To Typical Open Flame Combustion

Standard solutions of CBD, CBN and THC were dried down to solids and combusted. The recovery of THC (figure 4.16) was on average over 90% across three different concentrations (100, 200 and 400 µg/mL). Recovery of CBN (figure 4.17) was on average over 90% across initial concentrations of 20, 40, and 80 µg/mL. Recovery of CBD (figure 4.18) was on average over 80% across initial concentrations of 20, 40, and 80 µg/mL. The reason in the discrepancy for standard concentrations used for THC versus CBD and CBN was that in a typical high potency cannabis plant sample, these standard concentrations more accurately reflect the amount one would likely find in a plant sample (see figure 4.33 and figure 4.34). .

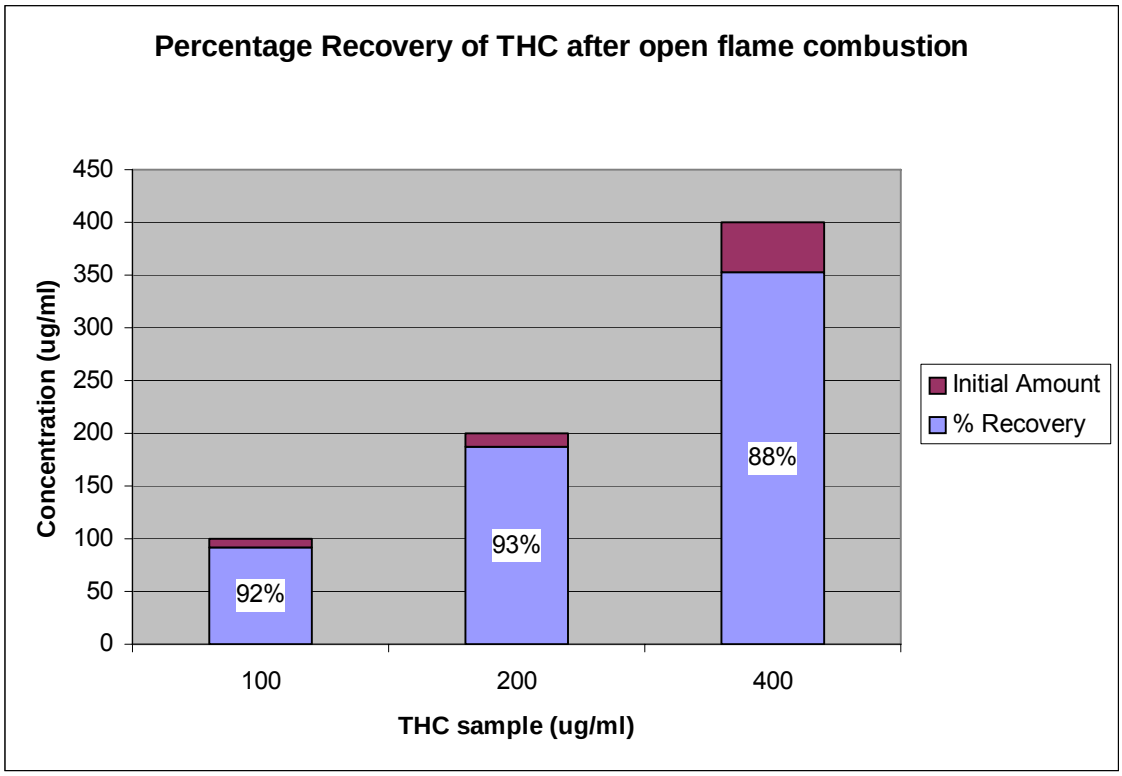


Figure 4.16 Percentage Recovery for THC across initial concentrations of 100, 200 and 400 µg/mL THC before and after combustion

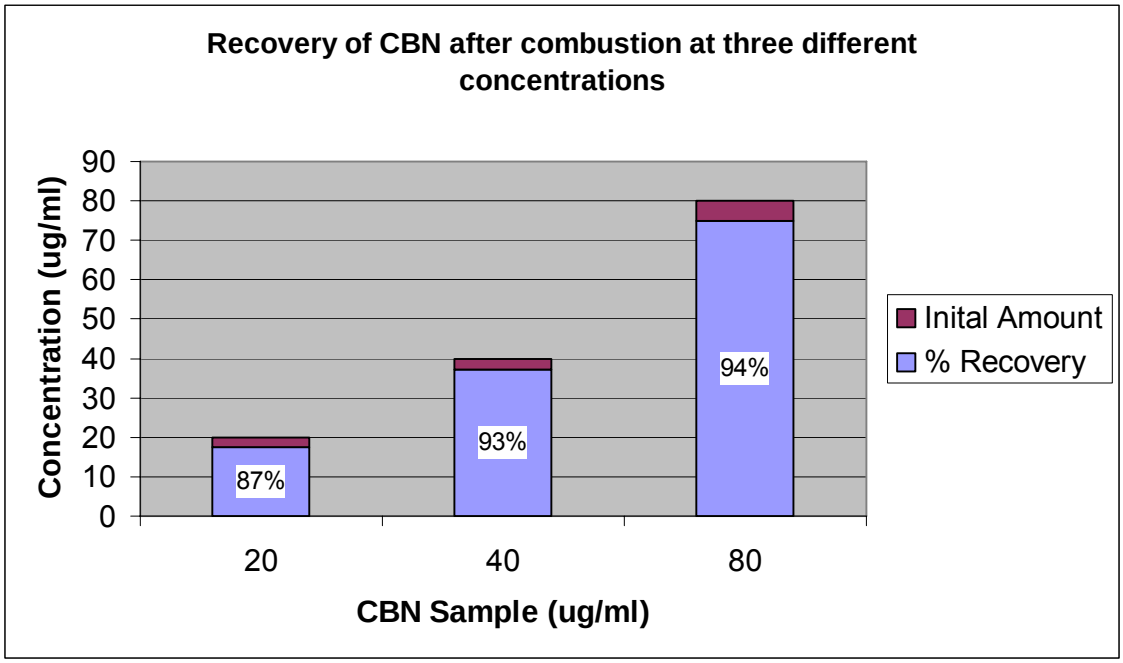


Figure 4.17 Percentage Recovery for CBN across initial concentrations of 20, 40 and 80 µg/mL CBN before and after combustion

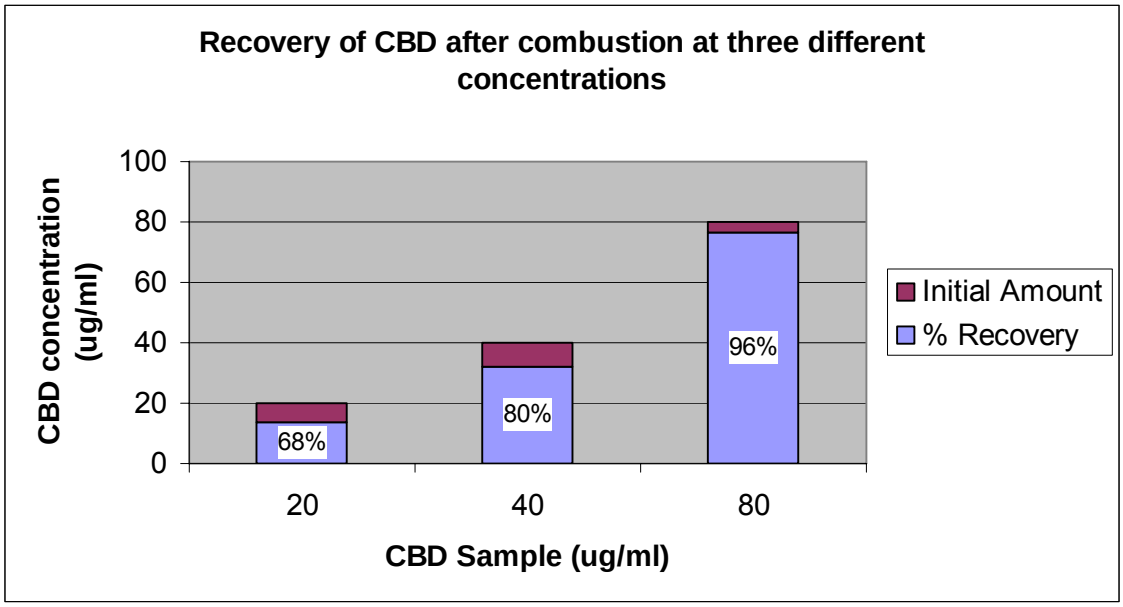


Figure 4.18 Percentage Recovery for CBD across initial concentrations of 20, 40 and 80 µg/mL CBD before and after combustion

4.5.1 Stability of THCA to Open Flame Combustion

It has been hypothesized that THCA is converted to THC upon combustion or exposure to heat. Table 4.13 is the initial amount of THCA analyzed and quantitated before combustion. The same samples were then combusted and analyzed using HPLC-UV to qualitatively and quantitatively describe the changes. Table 4.14 is the resulting amount of CBN, CBD, THC and CBC quantitated after combustion of the THCA standards. Note that due to the unavailability of a CBC standard, only the peak areas are displayed. in the table. It can clearly be seen that the recovery of THCA is below the limit of quantification. Furthermore, the recovery of THC falls in the range of 49-55% across the three different standard concentrations (113, 225 and 450 µg/mL) (figure 4.19). Two HPLC-UV chromatograms showing the before and after combustion of the

raw cannabinoid plant extract can be found in section 4.11 figure 4.33 and 4.34, respectively.

Table 4.13 Uncombusted Samples of THCA Standard (µg/mL)

SAMPLE	THCA	
	AMT	AREA
0203-10a 113 THCA liquid	116.3	3390394
0203-10b 113 THCA liquid	111.5	3252342
0203-10c 113 THCA liquid	120.9	3523232
AVERAGE	116.2	3388656
0203-11a 225 THCA liquid	246.3	7122012
0203-11b 225 THCA liquid	254.0	7342234
0203-11c 225 THCA liquid	245.7	7103454
AVERAGE	248.7	7189233.3
0203-12a 450 THCA liquid	465.8	13420859
0203-12b 450 THCA liquid	449.2	12945455
0203-12c 450 THCA liquid	451.9	13022344
AVERAGE	455.6	13129553

* Three different concentrations of THCA standard before combustion analyzed in triplicate on HPLC-UV.

Table 4.14 Combusted Samples of the THCA Standard (µg/mL)

	CBN		**THC**		**THCA**		**CBC**
	amt	area	amt	area	amt	area	area
0203-3b 113 THCA 3M	BLQ		29.7	778263	BLQ		1750754.8
0203-2b 113 THCA 3M	BLQ		45.7	1274934	BLQ		1589118.8
0203-1b 113 THCA 3M	BLQ		47.1	1316202	BLQ		1582425.3
AVERAGE			40.8	1123133			1640766.3
0203-6b 225 THCA 3M	6.3	351440	103.2	3059029	BLQ		1260623.4
0203-5b 225 THCA 3M	6.6	377001	105.2	3120689	BLQ		1254970.5
0203-4b 225 THCA 3M	4.8	240604	81.0	2368997	BLQ		1256369.0
AVERAGE	5.9	323015	96.5	2849571			1257320.9
0203-9b 450 THCA 3M	11.2	725051	173.5	5240320	BLQ		669418.3
0203-8b 450 THCA 3M	11.3	733056	179.0	5411495	BLQ		593086.4
0203-7b 450 THCA 3M	11.4	741931	198.2	6006438	BLQ		518554.0
AVERAGE	11.3	733346	183.6	5552751			593686.2
SAMPLE	**CBN**		**THC**		**THCA**		**CBC**
	amt	area	amt	area	amt	area	area
0203-3a 113 THCA TT	4.9	249234	34.1	915629	BLQ		BLQ
0203-2a 113 THCA TT	4.5	215986	14.5	306857	BLQ		BLQ
0203-1a 113 THCA TT	BLQ	BLQ	17.4	397373	BLQ		BLQ
AVERAGE	4.7	232610	22.0	539953			
0203-6a 225 THCA TT	BLQ		22.2	545302.6	BLQ		BLQ
0203-5a 225 THCA TT	BLQ		21.6	525346	BLQ		BLQ
0203-4a 225 THCA TT	BLQ		30.1	790700.5	BLQ		BLQ
AVERAGE			24.6	620449.7			
0203-9a 450 THCA TT	BLQ		63.5	1826402	BLQ		BLQ
0203-8a 450 THCA TT	BLQ		48.5	1362525	BLQ		BLQ
0203-7a 450 THCA TT	BLQ		36.9	1002162	BLQ		BLQ
AVERAGE			49.7	1397030			
TOTAL 450			**220.5**	**6554913**			
TOTAL 225			**121.1**	**3470021**			
TOTAL 113			**62.9**	**1663086**			

* Resultant concentrations of CBN, CBC and THC after combustion of THCA standard analyzed in triplicate on HPLC-UV.

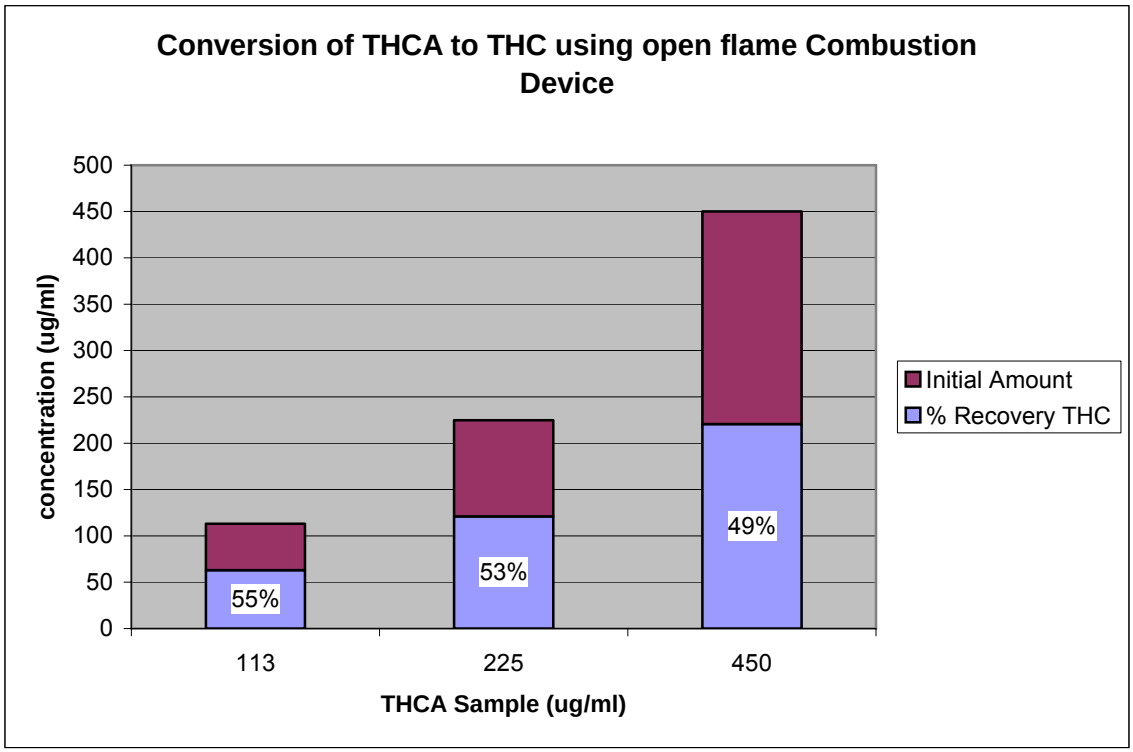


Figure 4.19 Percentage Recovery for THC across initial concentrations of 113, 225 and 450 µg/mL THCA before and after combustion.

4.6 Capacity of the SPE Method in a Serial Arrangement

Another experiment involved using 2 solid phase extraction cartridges in a serial arrangement to see if the first SPE was unable to capture all of the cannabinoids under vacuum after being combusted. Figure 4.20 shows the results of the HPLC-UV analysis of the first SPE cartridge. Again, approximately 50% of the THCA was converted to THC which is extracted from the first SPE cartridge. The second SPE cartridge (figure 4.21) shows that no cannabinoids were detected giving strength to the argument that the first SPE cartridge is capable of detecting and capturing virtually all of the cannabinoids in the smoke at this concentration.

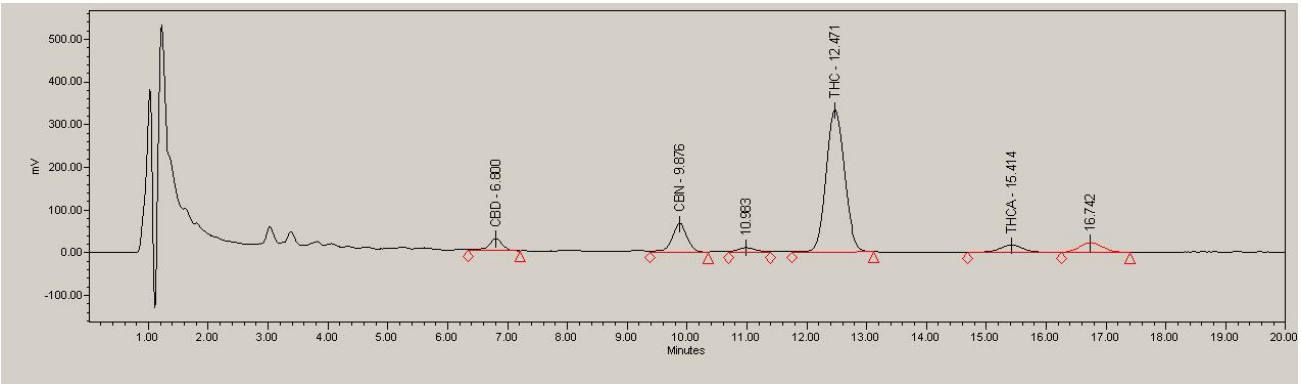


Figure 4.20 HPLC-UV chromatogram of cannabinoids from first SPE cartridge in a serial arrangement.

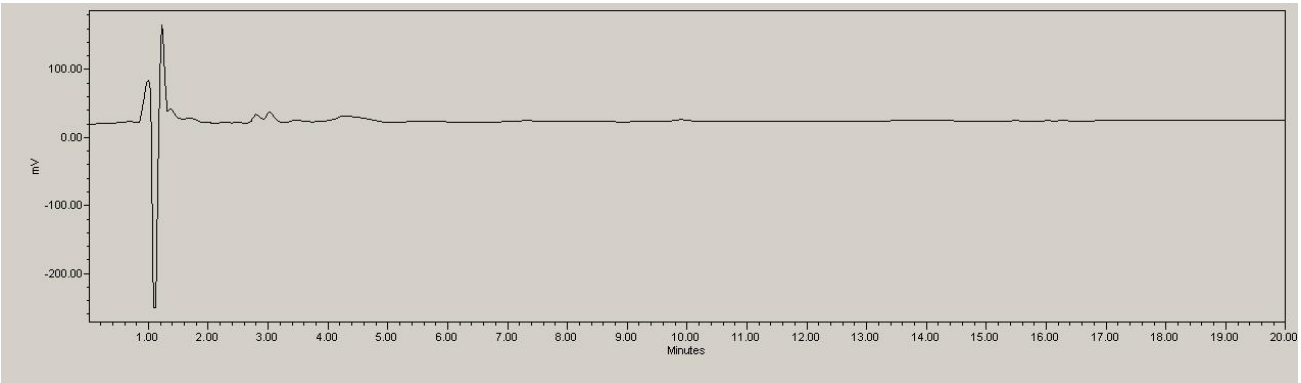


Figure 4.21 HPLC-UV chromatogram of cannabinoids from second SPE cartridge in a serial arrangement

4.7 Capacity of the SPE Method Using Plant Tissue

The capacity of the SPE extraction method was investigated. This was necessary to determine the sample load of the SPE cartridge as employed in the SPE combustion system.

Figure 4.22 shows that the SPE method for THC shows a linear increase in capacity as one increases the amount of plant material combusted from 5 mg to 10 mg. As the amount is increased further to 15 mg there is an apparent leveling off in the

cartridge capacity. For CBN (figure 4.23), the SPE method shows a linear increase in capacity as one increases the amount of plant material combusted from 5 mg to 10 mg. As the amount is increased further to 15 mg the cartridge still maintains it linearity in response. For CBD (figure 4.24) and CBC (figure 4.25), there is also a linear increase in the response shown between 5 and 10 mg plant samples. However, the leveling off at 15 mg is apparent. Therefore, based on all analytes in the standard plant samples, it appears that the SPE cartridge system should be not used with sample sizes greater then 10 mg of cannabis plant for the combustion and extraction system as the capacity for extraction is reached. Furthermore, the sensitivity appears to be very good at the 5 mg level and hence most of the experiments performed were carried out at this concentration when using raw plant samples.

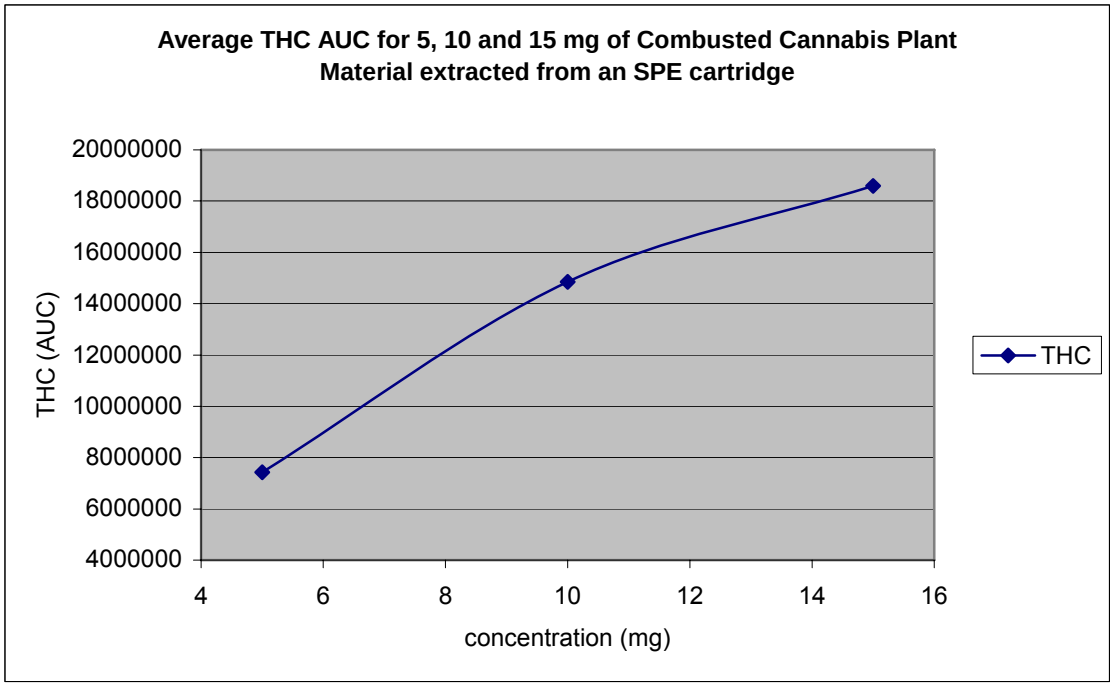


Figure 4.22 THC AUC for 5, 10 and 15 mg of combusted cannabis plant material extracted from 3M SPE cartridge

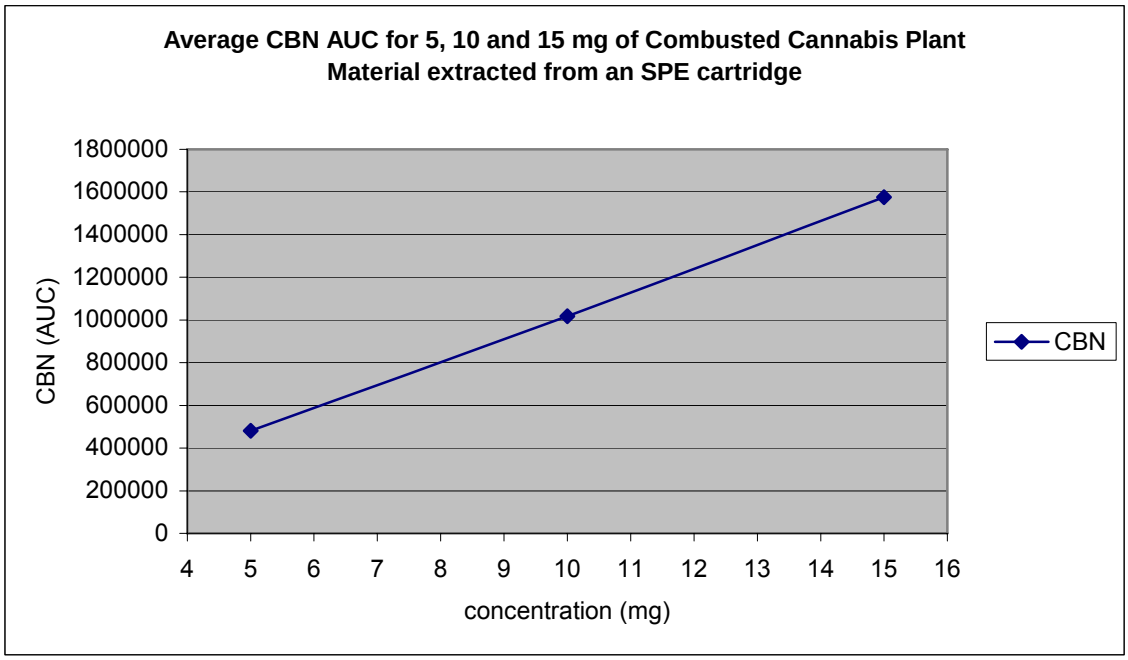


Figure 4.23 CBN AUC for 5, 10 and 15 mg of combusted cannabis plant material extracted from 3M SPE cartridge

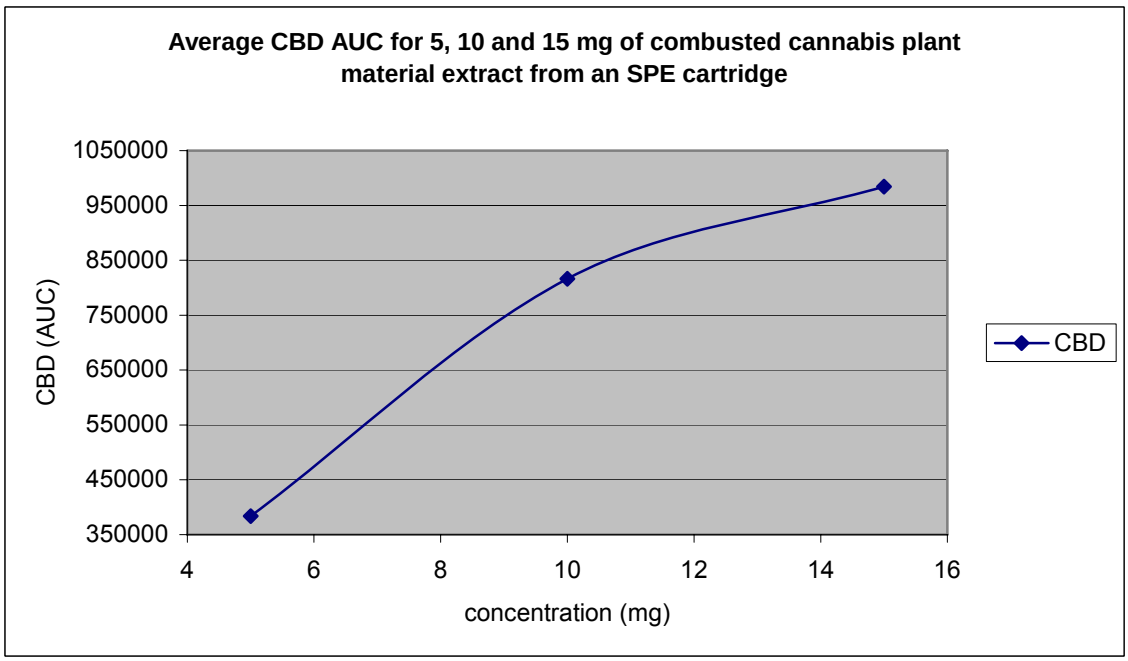


Figure 4.24 CBD AUC for 5, 10 and 15 mg of combusted cannabis plant material extracted from 3M SPE cartridge

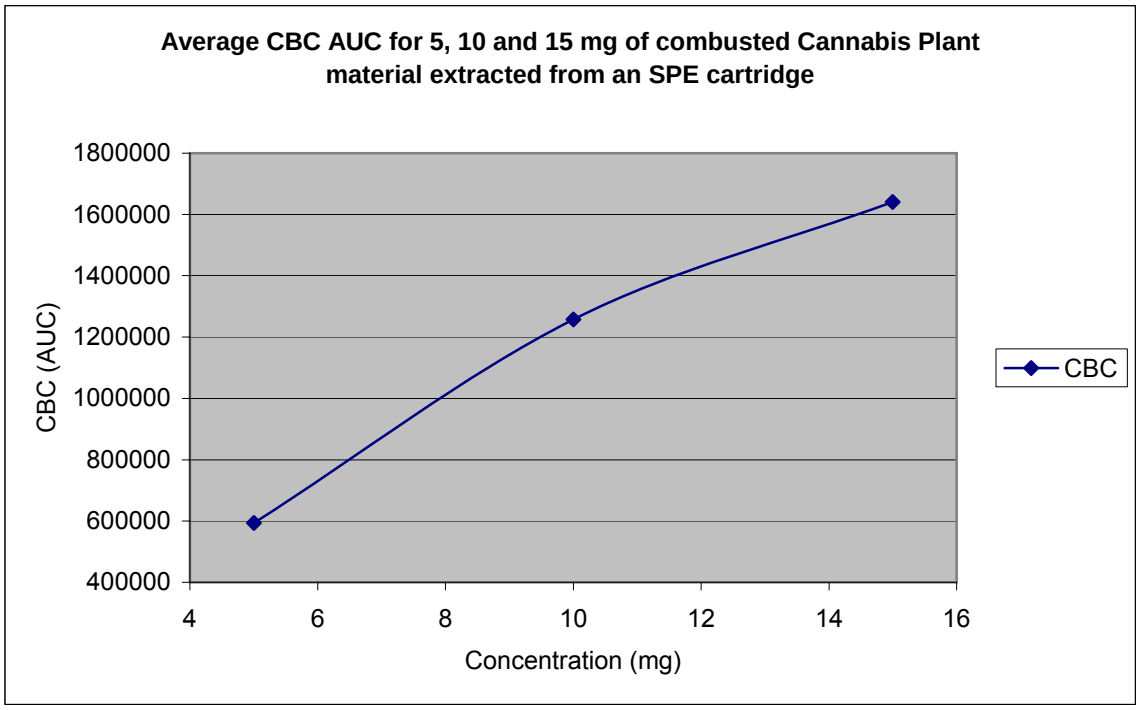


Figure 4.25 CBC AUC for 5, 10 and 15 mg of combusted cannabis plant material extracted from 3M SPE cartridge

4.8 Conversion of THCA to THC and Other Cannabinoids at Different Temperatures Held Over a Constant Time Frame

The transition of THCA to THC was investigated using the SPE method and combustion system. This particular experiment involved using a vacuum tight sealed glass vial and heating a known amount of plant material and then measuring the concentration of THCA/THC at predetermined temperatures. Each temperature range was held for 30 minutes. Table 4.15 and figure 4.26 show the results of this experiment. The results demonstrate that at 50 degrees celcuis, THCA was surprisingly stable and was highly recoverable at this temperature (no THC was detected in 3 of the 4 tests and the concentration of THCA stayed constant at 236.7 µg/mL). However, at 100 degrees celcius, THCA begins to decompose. At this temperature there is an almost equal

concentration of THCA and THC (87.26 µg/mL THCA compared to 110.45 µg/mL THC). This is interesting because when you go on to the next temperature threshold of 150 degrees celcius, there is an increase in the concentration of THC but a greater concentration of THCA is also destroyed (121.6 µg/mL THC and 11.55 µg/mL THCA). At 200 degrees, the only quantifiable compound is CBN. This is an important observation because it gives further weight to the hypothesis that cannabinoids are in fact very heat labile compounds and all of which eventually break down into their core elements when exposed to excessive heat.

Table 4.15 THCA conversion to THC and CBN at Different Temperatures held for 30 minutes.

THCA Conversion (µg/mL) to THC and CBN at Different Temperatures held for 30 minutes					
TEMP 'C	**SAMPLE**	**CBD**	**CBN**	**THC**	**THCA**
50	1	BLQ	BLQ	BLQ	237.86
	2	BLQ	5.94	BLQ	236.47
	3	BLQ	BLQ	13.07	235.13
	4	BLQ	BLQ	BLQ	237.50
	AVERAGE		**5.94**	**13.07**	**236.74**
100	5	BLQ	5.42	134.83	88.37
	6	BLQ	BLQ	104.74	86.62
	7	BLQ	BLQ	100.97	86.41
	8	BLQ	BLQ	101.26	87.63
	AVERAGE		**5.42**	**110.45**	**87.26**
150	1	BLQ	10.12	112.91	BLQ
	2	BLQ	10.43	119.01	10.24
	3	BLQ	10.50	123.26	BLQ
	4	12.72	10.95	131.21	12.86
	AVERAGE		**10.50**	**121.60**	**11.55**
200	1	BLQ	51.95	BLQ	BLQ
	2	BLQ	54.88	13.13	BLQ
	3	BLQ	53.09	12.23	BLQ
	4	BLQ	56.41	BLQ	BLQ
	AVERAGE		**54.08**	**12.68**	
250	1	BLQ	11.01	BLQ	BLQ
	2	BLQ	13.81	BLQ	BLQ
	3	BLQ	20.34	BLQ	BLQ
	4	BLQ	15.34	BLQ	BLQ
	AVERAGE		**15.13**		

**** The initial THCA concentration was 240 µg/mL. The Initial THC concentration was 12.3 µg/mL**

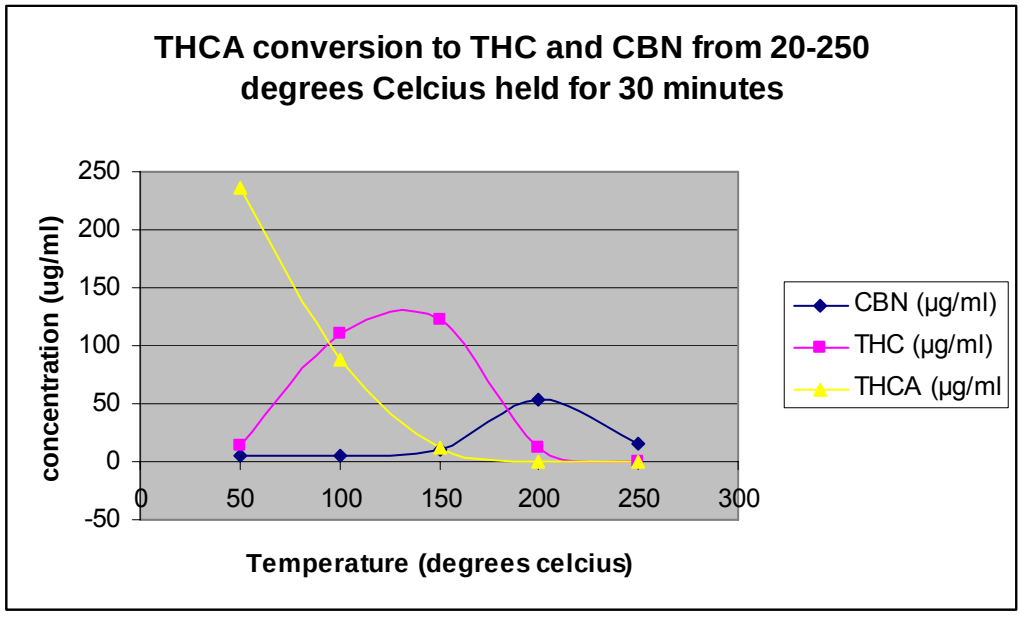


Figure 4.26 THCA conversion to THC and CBN from 20-250 degrees celcius at different temperatures held for 30 minutes.

An interesting observation is the 100-degree threshold. There has been a large amount of THC already produced from the THCA. However, there is still a relatively large amount of THCA still quantifiable. It is somewhere between this temperature and 50 degrees that is the flash point of the reaction. Further investigations could prove interesting. Also what is quite fascinating is at the 200 degree mark, CBN is the most abundant molecule. Lastly, at the 250 degree mark virtually all of the cannabinoids are at or below the LLQ.

4.9 The Conversion of THCA to Related Cannabinoids as a Function of Time at a Single Temperature

The rate of decomposition of THCA to THC at a set temperature was investigated. A temperature of 200°C was chosen as it was a temperature that demonstrated both

qualitative and quantitative changes in the cannabinoids over a short period of time before thermal destruction set in. Table 4.16 and figure 4.27 show that after approximately 1 minute at 200°C, most of the THCA has been converted to THC. For the next 4 minutes, the THC concentrations stay virtually identical despite being heated at 200°C. Simultaneous to the conversion of THCA to THC is the increase in CBN concentration that slowly begins to rise after 2 minutes. After 5 minutes, THC concentrations start to decrease and continue to decrease rapidly until there is virtually none left at 25 minutes. At the same time, CBN concentrations continue to increase right up until the 25 minute mark. The increase in CBN concentration is most likely formed from THC. Figure 4.28 shows the chemical structural change from THC to CBN. The only change is the loss of the 2 hydrogen atoms on the CBN molecule resulting in the formation of an aromatic ring.

Table 4.16 Conversion of THCA to THC over 25 minute period at 200 degrees celcius

THCA conversion to THC over 25 minute period at 200 degrees celcius					
Time	SAMPLE	CBN (µg/mL)	THC (µg/mL)	THCA (µg/mL	% Conversion
0	Initial	BLQ	12.3	260	
1					
	1	BLQ	149.40	26.80	57.46
	2		153.00	24.00	58.85
	3		152.00	23.00	58.46
	AVERAGE		151.47	24.60	58.26
2					
	4	BLQ	152.00	<10	58.46
	5		155.00	<10	59.62
	6		151.00	<10	58.08
	AVERAGE		152.67		58.72
3					
	7	7.80	153.00	<10	58.85
	8	6.70	151.00	<10	58.08
	9	8.20	154.00	<10	59.23
	AVERAGE	7.57	152.67		58.72
4					
	10	11.70	153.00	<10	58.85
	11	10.70	150.00	<10	57.69
	12	11.40	154.00	<10	59.23
	AVERAGE	11.27	152.33		58.59
5					
	13	27.02	157.00	<10	60.38
	14	26.90	159.00	<10	61.15
	15	28.30	158.00	<10	60.77
	AVERAGE	27.41	158.00		60.77
10					
	16	39.40	84.40	<10	32.46
	17	40.80	83.30	<10	32.04
	18	38.90	86.30	<10	33.19
	AVERAGE	39.70	84.67		32.56
15					
	19	51.30	54.50	<10	20.96
	20	50.80	52.10	<10	20.04
	21	51.90	55.50	<10	21.35
	AVERAGE	51.33	54.03		20.78
20					
	22	56.04	38.20	<10	14.69
	23	57.30	36.30	<10	13.96
	24	55.40	39.40	<10	15.15
	AVERAGE	56.25	37.97		14.60
25					

	25	64.20	<10	<10	N/A
	26	63.60	<10	<10	
	27	65.20	<10	<10	
	AVERAGE	64.33			

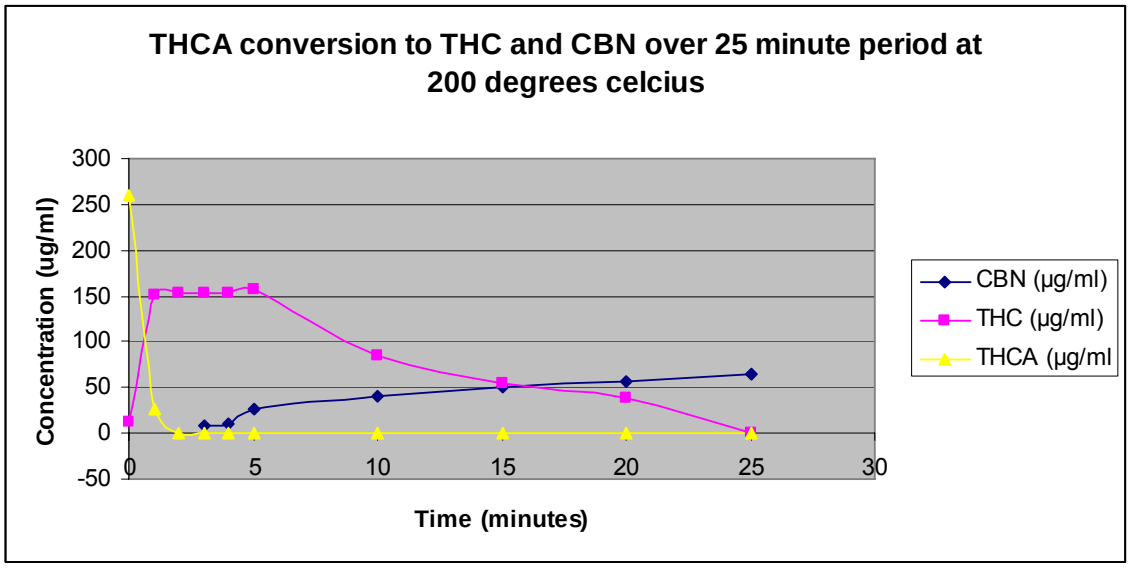
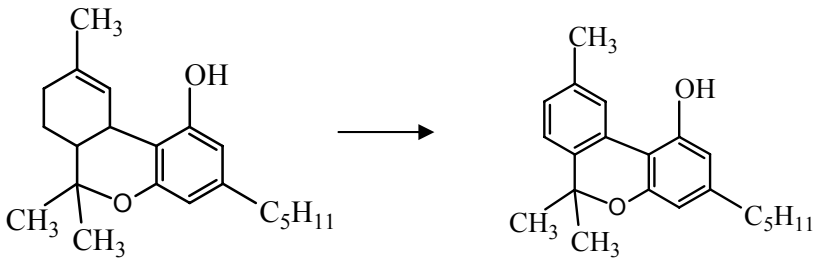


Figure 4.27 THCA conversion to THC and CBN over a 25 minute period at 200 degrees celcius



THC CBN

Figure 4.28 Chemical Structure of THC and CBN. Note the loss of 2 Hydrogen ions on the upper left ring of CBN, which is the only difference between these two compounds.

4.10 Other Products from the Thermal Decomposition of THCA

The lack of quantitative conversion of THCA to THC may suggest that other compounds not identified during HPLC-UV may account for the apparent loss of THCA. Consequently, samples were analyzed by LC/MS to help identify if other cannabinoid type compounds were present after combustion. All results were obtained using both positive and negative electrospray ion modes. All samples were infused at a rate of 1 mL/min. Figure 4.29 is a full scanning negative ion mass spectrum of the methanolic extract of cannabis plant material. Here a predominant ion at 357 m/z can be seen. This ion corresponds to the M-H of THCA. No other significant cannabinoid related ions are present in the mass spectrum.

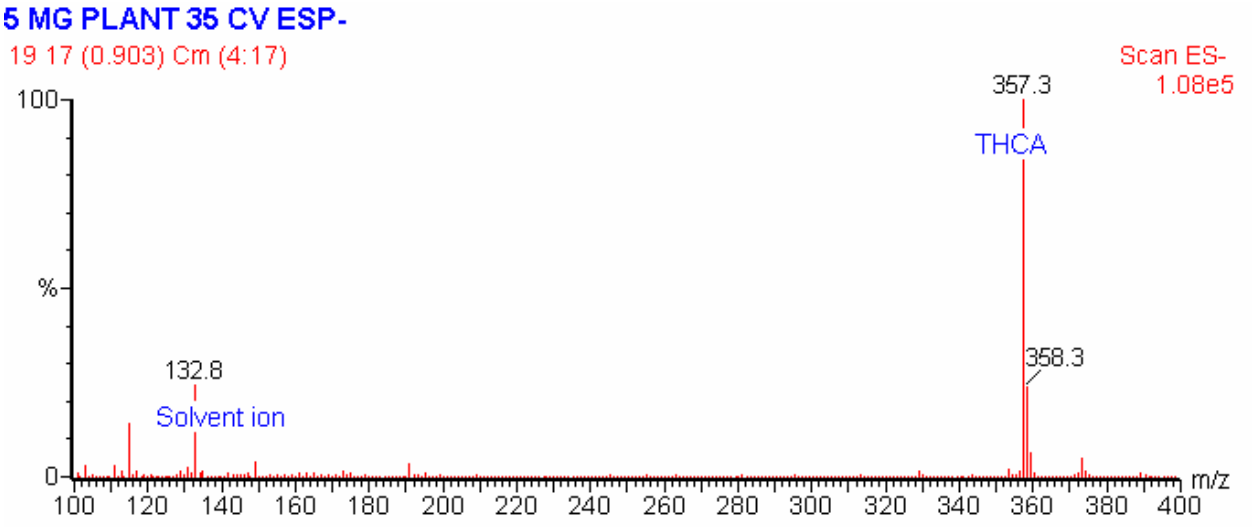


Figure 4.29 Full scanning negative ion mass spectrum of the methanolic extract of cannabis plant material infused into the mass spectrometer.

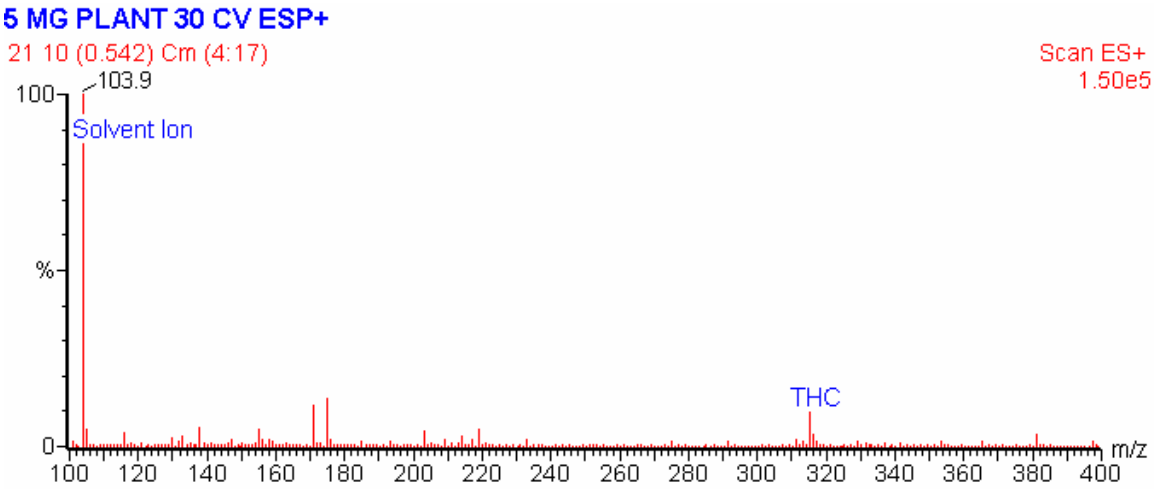


Figure 4.30 Full scanning positive ion mass spectrum of the methanolic extract of cannabis plant material infused into the mass spectrometer.

Figure 4.30 is the analogous sample analyzed in positive ion mode where only ions related to THC (M+H of THC) 315 molecular weight can be seen. THCA cannot be seen in this ion chromatogram because the acid entity does not protonate easily in an organic solvent. Hence acids are better analyzed under negative ion mode as demonstrated above.

Performing the same experiment now after the plant extract has been combusted gives the following results (Figure 4.31):

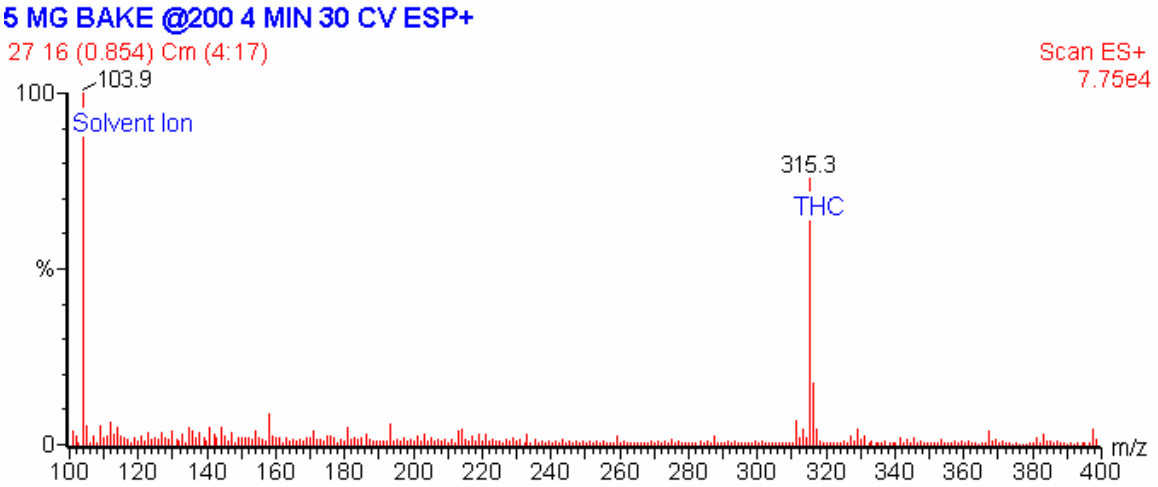


Figure 4.31 Full scanning positive ion mass spectrum of the methanolic extract of combusted cannabis plant material infused into the mass spectrometer.

Figure 4.31 demonstrates under positive ion mode a large increase in the intensity of the THC peak. Again, there are no extraneous peaks that could account for the 40-50% loss of THCA.

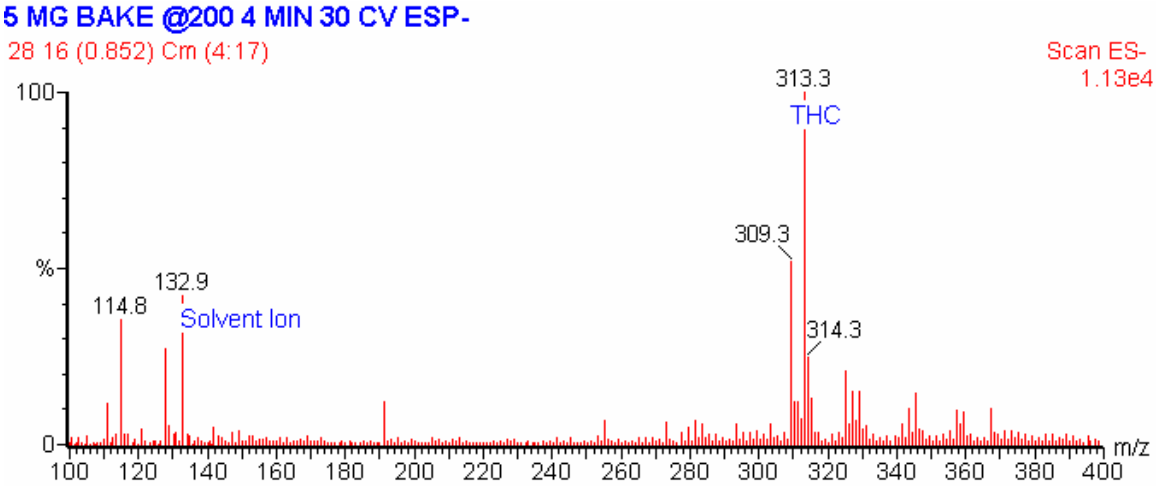


Figure 4.32 Full scanning negative ion mass spectrum of the methanolic extract of combusted cannabis plant material infused into the mass spectrometer.

Figure 4.32 shows that after combustion under negative ion mode, there is virtually no THCA detectable. Also notice, that although negative ion mode shows much less

sensitivity for THC, the quantity of THC is so great after combustion that it can be detected now.

4.11 Chemo-Botanic Profiling of a Typical Raw Cannabis Plant Extract Before and After Combustion

Chemo botanic profiling of a typical raw cannabis plant extract yielded the following chromatogram (Figure 4.33):

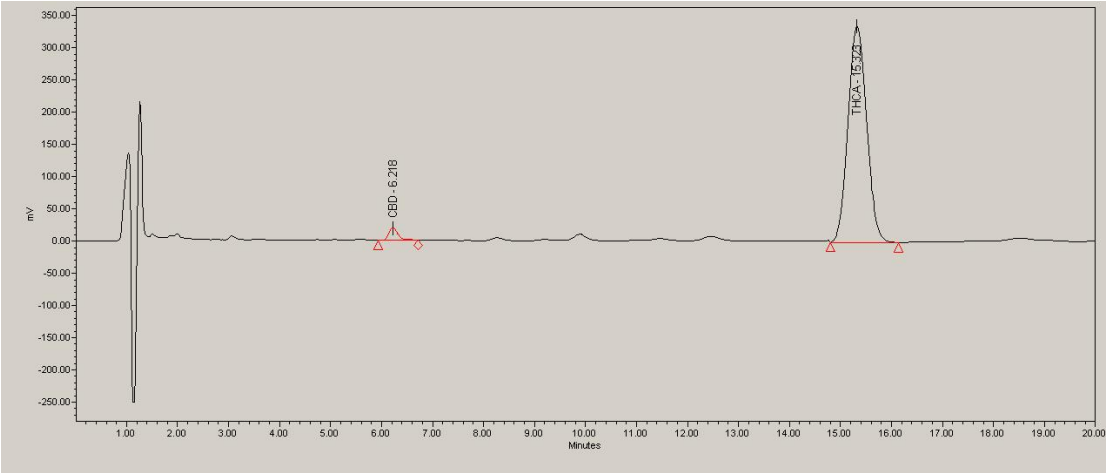


Figure 4.33 HPLC-UV chromatogram of cannabinoids found in typical high potency marihuana plant.

Figure 4.33 shows the major cannabinoid present in a typical plant extract is THCA with very minor quantities of CBD, CBN, and THC. The initial concentration of THCA in this sample was estimated to be 830 µg/mL. If this plant sample is combusted and the smoke analyzed, the following chromatogram was obtained (figure 4.34):

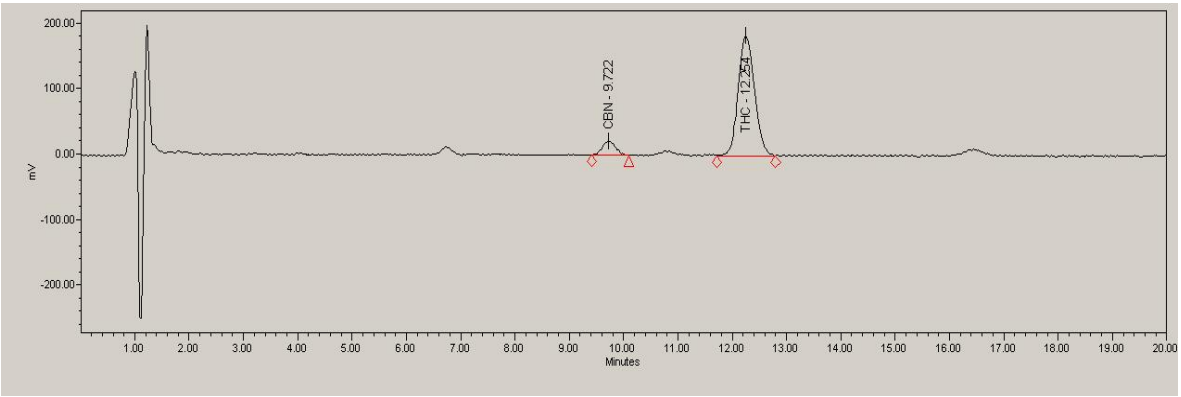
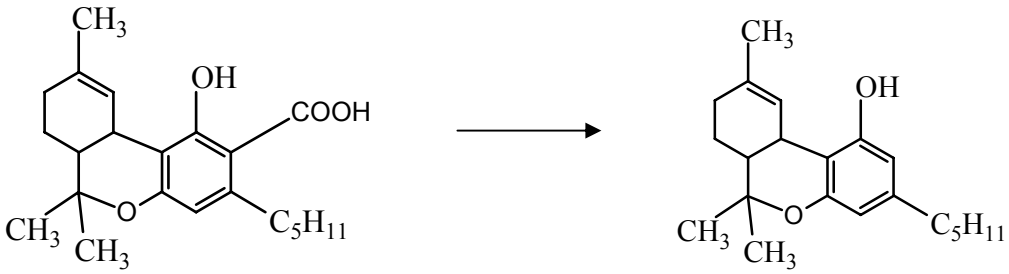


Figure 4.34 HPLC-UV chromatogram of cannabinoids found in a typical high potency marihuana plant after combustion.

Figure 4.34 shows that there is now a very small quantity of the THCA and a large increase in quantity of THC (420 µg/mL). There was also a small increase in the CBN concentration. The increase in THC concentration only accounts for approximately 50% of the initial THCA concentration. The remainder of the THCA is unaccounted for in this conversion process. Figure 4.35 is the molecular structures of THCA and THC. The only difference is the carboxylic acid group on the number two carbon.



THCA THC

Figure 4.35 The conversion of THCA TO THC

4.12 Other Samples with Differing Concentrations of THCA

For much of the research shown above, a standardized sample marihuana plant has been used in order to keep the number of variables in the experiments to a minimum. However, below are two other standardized samples of marihuana plant that have known differing concentrations of THCA. A certificate of analysis for sample 1502 states that it contains 3% THCA and 0.08% THC. Table 4.17 and figure 4.36 show the concentration of THC found after combustion is equal to 2% THC or 66% of the original concentration of THCA.

Table 4.17 Combusted Marihuana plant sample 1502 (µg/mL) Initial concentration of THCA before combustion was 154.4 µg/mL

1502 SAMPLE				
TEST TUBE WALLS MEOH RINSE				
SAMPLE	CBN	THC	THCA	CBC
1		63.11		735802
2		75.21		879370
3		59.96		688951
4		59.23		689092
5				
AVERAGE		64.38		748303.8
STDEV		7.41		90117.4
TEST TUBE WITH 3M ATTACHED 3M RINSE				
SAMPLE				CBC
1	2.97	18.92		203197.5
2		13.81		
3	3.17	23.62		257827
4	3.67	28.16		284996.3
5	6.19	103.07		1292538
AVERAGE	4.00	37.52		509639.7
STDEV	1.49	37.03		523039.5
TOTALS	**4.00**	**101.90**		**1257943**

Certificate of analysis for sample 1505 shows that this sample contained 10% THCA and 0.1% THC. Upon combustion (table 4.18 and figure 4.36) the concentration of THC in the sample works out to be 5.7% which is 57% of the original concentration of THCA. This gives further evidence that the conversion of THCA to THC is in the order of 50-60%. Here there is further evidence to support the 50-60% percent conversion hypothesis as it has now been shown across different samples of differing concentrations of THCA that the ratio of THCA to THC converted remains similar.

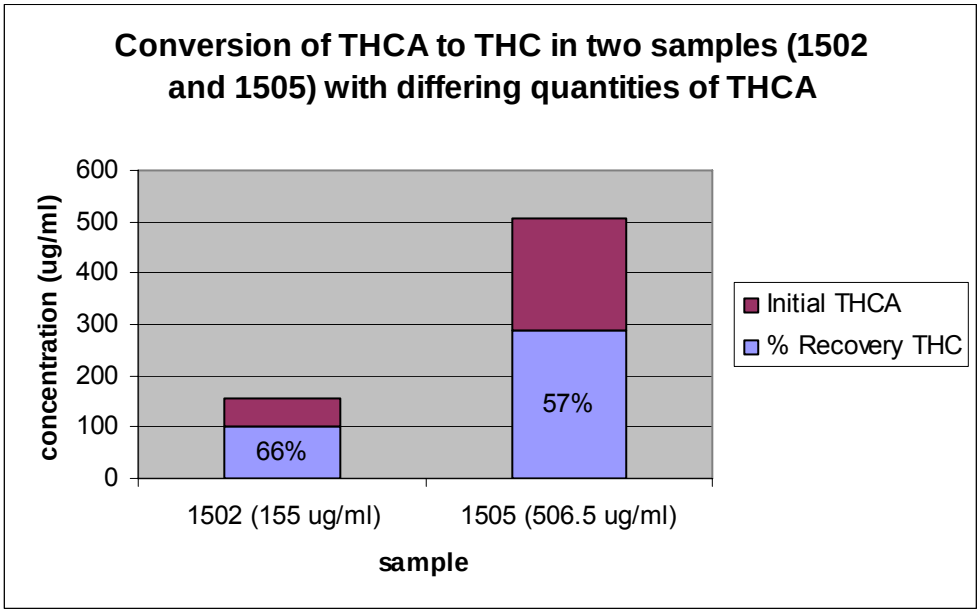


Figure 4.36 Conversion of THCA to THC in two diverse cannabis samples #1502 and #1505 that contain differing quantities of THCA.

Table 4.18 Combusted Marihuana plant sample 1505 (µg/mL). Uncombusted THCA concentration was 506.5 µg/mL

1505 SAMPLE					
TEST TUBE WALLS METHANOL RINSE					
SAMPLE	CBD	CBN	THC	THCA	CBC
1	12.15	4.55	254.41		865461.2
2	12.39	4.54	254.50		871730.1
3	9.77	3.68	209.26		713399
4	10.30	4.10	215.96		731104.1
5	11.41	4.14	235.95		788290.5
AVERAGE	11.20	4.20	234.01		793997
STDEV	1.14	0.35	21.08		73541.95
TEST TUBE WITH 3M ATTACHED 3M RINSE					
SAMPLE					
1		3.24	46.34	20.74	
2		3.31	30.44	16.41	
3		5.03	72.22	27.73	217606
4		4.22	65.55	20.72	212233.2
5		3.67	58.96	19.58	
AVERAGE		3.90	54.70	21.04	214919.6
STDEV		0.74	16.59	4.13	3799.139
TOTALS	**11.21**	**8.11**	**288.72**	**21.04**	**1008917**

4.13 Comparison of Different Smoking Devices - The Analysis of Different Devices Used for Inhaling Marihuana

As well as determining the quantitative and qualitative profile of marihuana after combustion has occurred, an experiment was also carried out to determine if there are any differences in the amount of cannabinoids present in smoke delivered by different types of pipes. As well, a common vaporizer and water bong were also examined to determine the cannabinoid profiles arising from these devices and reaching the outlet orifice under a specified vacuum. A random sample of smoking devices was obtained from the RCMP and is shown in Figures 4.37-41. The vaporizer was the only purchased device that was obtained from an online internet retailer.

Figure 4.37 PIPE #1 donated by RCMP for Marihuana Analysis

Table 4.19 Combustion data for Pipe #1 (µg/mL)

SAMPLE	CBD	CBN	THC	THCA	CBC
1	19.22	11.49	324.64	11.38	679089
2	22.38	9.61	381.74	9.23	859607
3	21.32	9.86	347.80	12.92	743461
4	21.19	11.52	354.42	2.49	751595
5	21.13	8.86	352.83	14.00	779526
6	23.41	11.56	432.65	13.57	993654
7	20.56	11.14	401.35	11.32	828459
8	22.98	10.36	382.12	14.49	823188
9	22.97	9.36	378.73	38.85	801149
10	22.53	11.75	399.10	10.93	827965
AVERAGE	21.77	10.55	375.54	15.19	808769
SD	1.31	1.070794	31.44231	9.412854	83662.99

Figure 4.38 PIPE #2 donated by RCMP for Marihuana Analysis

Table 4.20 Combustion data for Pipe #2 (µg/mL

SAMPLE	CBD	CBN	THC	THCA	CBC
1	18.01	10.71	316.74		859607.00
2	25.12	15.42	421.36		659043.97
3	24.11	13.31	410.08		900141.00
4	23.54	11.48	404.75	9.24	861888.88
5	21.91	11.47	368.70		768854.53
6	23.08	11.84	397.63		853190.00
7	21.24	11.77	355.00		725839.50
8	21.53	13.22	370.82	8.56	832271.60
9	22.30	11.95	374.83		806647.57
10	20.28	10.44	349.06		742566.06
AVERAGE	22.11	12.16	376.90	8.90	801005.01
STDEV	2.04	1.47	32.09	0.48	75242.76

Figure 4.39 PIPE #3 donated by RCMP for Marihuana Analysis

Table 4.21 Combustion data for Pipe #3 (µg/mL)

SAMPLE	CBD	CBN	THC	THCA	CBC
1	15.01	7.44	239.79	15.00	695044
2	17.43	8.48	284.08	14.73	803362
3	13.21	8.18	221.08	11.15	572194
4	13.25	6.43	208.17	14.10	572808
5	16.12	7.26	256.93	12.62	695212
6	13.40	7.53	216.09	10.39	582313
7	14.20	7.08	224.86	12.43	631231
8	15.86	8.87	267.83	17.42	737772
9	17.06	9.25	281.60	13.99	801427
10	15.81	8.07	255.13	15.96	726035
AVERAGE	15.14	7.86	245.55	13.78	681739
STDEV	1.56	0.86	27.53	2.16	88744

Figure 4.40 PIPE #4 donated by RCMP for Marihuana Analysis

Table 4.22 Combustion data for Pipe #4 (µg/mL)

SAMPLE	CBD	CBN	THC	THCA	CBC
1	14.84	7.95	241.85	12.11	648362
2	10.63	5.24	159.35	7.91	452605
3	14.24	7.33	225.22	10.42	634415
4	10.97	6.24	167.78	10.74	503688
5	9.24	6.66	193.81	11.87	578656
6	10.68	6.57	217.11	12.17	626717
7	10.70	5.50	153.12	9.88	448328
8	13.12	6.31	208.10	10.80	591864
9	14.98	7.60	241.10	9.10	701760
10	15.05	6.74	243.95	15.85	714205
AVERAGE	12.45	6.61	205.14	11.08	590060
STDEV	2.23	0.85	35.01	2.15	94970

Figure 4.41 WATER BONG donated by RCMP for Marihuana Analysis

Table 4.23 Combustion data for Water Bong (µg/mL)

SAMPLE	CBD	CBN	THC	THCA	CBC
1	19.24	11.12	180.43	BLQ	871114.5
2	17.93	8.44	168.92	5.04	793895.2
3	14.55	11.56	138.41	8.18	668740.8
4	16.19	8.76	161.32	5.71	750145.7
5	13.53	8.24	172.77	9.87	772064.9
6	12.32	6.11	131.06	4.75	652393
7	13.68	7.62	140.57	2.93	682534.6
8	17.56	8.65	164.51	4.01	812841.5
9	16.05	7.68	156.03	4.31	747653
10	12.73	8.48	164.62	6.13	754746.5
AVERAGE	15.38	8.66	157.86	5.66	750613
SD	2.37	1.61	16.18	2.17	68099.19

Figure 4.42 VAPORIZER purchased from online retailer for Marihuana Analysis

Table 4.24 Combustion data for Vaporizer taken from walls of vaporizer (µg/mL)

SAMPLE	CBN	THC	THCA	CBC
1 jar	5.71	103.56		250972.5
2 jar	3.30	124.24		
3 jar	2.14	105.08		259006
4 jar	4.07	90.39		249334
5 jar	5.43	107.72		262297
6 jar	6.05	139.09		309352.5
7 jar	7.43	154.76		378535.5
8 jar	3.63	109.39		298089
9 jar	4.52	120.63		295605.5
10 jar	4.31	92.21		213671
AVERAGE	4.66	114.71		
STDEV	1.53	20.32		

Table 4.25 Combustion data for Solid Phase Extraction column taken from Vaporizer outlet (µg/mL)

SAMPLE	CBN	THC
1 3M SPE	8.69	90.26
2 3M SPE	9.80	62.45
3 3M SPE	11.80	62.81
4 3M SPE	16.40	33.75
5 3M SPE	26.04	82.57
6 3M SPE	12.49	78.99
7 3M SPE	13.04	37.62
8 3M SPE	10.87	44.77
9 3M SPE	13.00	38.11
10 3M SPE	11.35	54.18
AVERAGE	13.35	58.55
STDEV	4.92	20.31

Upon examination of the data above, it can clearly be seen that only a few observations can be made. First of all, the cannabinoid profiles of all the pipes and the water bong are very similar. As well, the amount of cannabinoids captured by the extraction system is virtually the same in the pipes and water bong. However, what is interesting is how poorly the vaporizer performs in comparison to the pipes or the water bong. The vaporizer has significantly less THC reaching the extraction orifice. More importantly, most of the THC in the vaporizer is adsorbing onto the glass enclosure. The following conclusion could be made that due to the increased dead space in the vaporizer, the vacuum level is insufficient to pull the cannabinoids out of the vaporizer and into our extraction system. The results may be improved if a stronger vacuum was used. In addition, it is conceivable that upon prolonged use of the vaporizer the adsorptive sites on the glassware will undoubtedly become saturated. This "seasoning"

of the device may in fact improve its performance as measured by the amount of deliverable cannabinoids to the extraction systems.

5 CONCLUSION

Over the last few years there has been a rebirth of research focused around marihuana and its metabolites. Many investigations are under way to study the vast pharmacological properties of cannabinoids since it has been definitively shown that endogeneous cannabinoids exist in human beings. Furthermore, cannabinoid receptors have also been identified that are beginning to illustrate the complex puzzle that cannabinoids can play in vivo. This research project was undertaken with the premise of doing an analysis of the combusted cannabis plant product. The first hypothesis was to determine if the cannabinoid profile of a typical marihuana plant changes upon combustion. With that being said, a series of experiments were completed to construct a crude smoking machine that would allow the researcher to capture and analyze the smoke of the combusted cannabis plant. These data could then be compared to data that could be generated by analyzing the same plant sample on a HPLC-UV system using a known method for qualitatively analyzing and quantitatively analyzing four major cannabinoids: CBD, CBN, THC and THCA. A comparison was made comparing the cannabinoid profiles between the combusted plant sample and the raw plant sample. It was shown that the cannabinoid profile between the raw plant extract and the combusted plant extract did in fact change dramatically. The raw plant extract showed a large amount of THCA with very small quantities of CBD, CBN and THC. Upon combustion, the THCA peak completely disappeared and there was a large increase in the size of the THC peak and a smaller increase in the amount of the CBN peak. The CBD peak did not change considerably. This change in the chromatogram profile led to another hypothesis that the THCA is converted to THC in large quantities with some of

it also being converted to CBN. It may or may not be directly converted to CBN or it may be converted to THC first and then the THC is converted to CBN. A number of experiments were carried out to determine the thermal stability of each of the cannabinoids. This was important, as it would hopefully provide some important information as to which cannabinoids were most resistant to thermal breakdown. Known quantities of CBD, CBN, THC and THCA standards were all analyzed at 3 different concentrations and were combusted using the smoking machine. Each analyte was extracted as above and its percentage recovery versus the initial amount of standard was noted. For CBD, the percent recovery for the 20, 40 and 80 μg/mL samples was 68%, 80% and 96%. For CBN the percent recovery for the 20, 40 and 80 μg/mL samples was 87%, 93% and 94%. For THC the standard concentrations that were used were much higher since it was found in much higher concentrations after combustion. The standard concentrations for THC were 100, 200 and 400 μg/mL. The percent recovery after combustion for each concentration was 92%, 93% and 88%, respectively. Finally, THCA standard was analyzed and combusted at a concentration of 113, 225 and 450 μg/mL. As expected after combustion, virtually all of the THCA was undetectable; however a large amount of THC was now appearing. The amount of THC that appeared gave a recovery of 55%, 53% and 49% compared to the original THCA concentrations. These were exciting data as they showed that THCA conversion to THC may not be a 100% conversion reaction. More experiments were carried out that were designed to further test the limits of the smoking machine and corresponding extraction system. The capacity of the solid phase extraction system for products of cannabis combustion demonstrated that between 5-10 mg of plant material did not saturate the process when

the combustion products were analyzed for CBD, CBN, THC and CBC. For each analyte, the results all followed the same trend: At 5 mg of sample processed by the smoking machine a strong response was noted. At 10 mg of sample, a corresponding doubling of response was noted. At 15 mg of sample, an apparent saturation of the solid phase extraction cartridge began to appear in all of the data as there was no longer a complete linear increase in adsorption. There was an increase but it was less then the 5 to 10 mg increase.

Further validation of the designed smoking machine's ability to accurately capture combusted cannabinoids involved using a second solid phase extraction and placing it in a serial arrangement with the first solid phase extraction cartridge. The hypothesis behind this experiment was to show that virtually all of the combusted cannabinoids were being detained on the first solid phase extraction cartridge and very little if any were making it past the first solid phase extraction cartridge. Analyzing the contents of both cartridges clearly showed that if any cannabinoids were reaching the second SPE cartridge, their levels were below the LLQ of our HPLC-UV analytical system.

A triple quadrapole mass spectrometer was used to verify if other products of combustion could be seen that were not showing up on the UV detector. This experiment entailed taking the extracted combusted cannabis plant samples and injecting them using direct infusion on to the mass spectrometer. The mass spectrometer was set to electrospray positive and negative ion modes. Samples of combusted CBD, CBN, THC and THCA were analyzed in an attempt to detect if there were any other combustion products that were being formed that may have not been detected by the HPLC-UV system. After a thorough analysis, it was determined that no other

extemporaneous compounds were being formed from the conversion of THCA to THC that could account for the 40-50% loss of analyte. It has been hypothesized that the inexplicable loss of analyte is most likely being broken down into the core elements of carbon and hydrogen or as suggested by the investigators into polymeric material that is not detectable (either not ionized or chromatographically not detectable).

In order to try and follow the conversion of THCA to THC, a high temperature programmable oven was used. This experiment did not use a smoking machine but instead a series of pre-defined temperatures. THCA was heated at 50, 100, 150, 200 and 250°C for a period of 30 minutes. It was anticipated that by doing so, the conversion of THCA to THC could be examined more carefully and perhaps shed some insight into its non quantitative nature. The results showed that at 50°C, THCA is surprising stable and was highly recoverable at this temperature. However, at 100°C, conversion rapidly ensues. At this temperature there is an almost equal amount of THCA and THC. This is interesting because when you go on to the next temperature threshold of 150°C, there is an increase in the amount of THC but a greater amount of THCA is also destroyed. At 200°C, the only quantifiable compound is CBN. This is an important insight because it gives further strength to the hypothesis that cannabinoids are in fact very heat labile compounds and all of which eventually break down into their core elements when exposed to excessive heat.

The high temperature oven was used again for more tests. However, this time, temperature was held constant and the dependent variable was time. A temperature of 200°C was chosen and time was measured in increments of one to five minutes and then from five minutes every five minutes to 25 minutes. In the first minute, virtually all of the THCA is converted to THC. For the next 5 minutes, the THC levels stay fairly

constant and then after 5 minutes they start to decrease with a subsequent increase in CBN levels. This was very interesting as it strongly suggests that CBN is not converted directly from THCA but rather is converted from THC. The CBN levels continued to increase right up until the 25 minute mark at which point they began to drop due to thermal decomposition.

A number of devices used for smoking marihuana were also tested and analyzed. A series of pipes borrowed from the RCMP that had been confiscated over the years were hooked up to our smoking machine and the resultant cannabinoid profiles were evaluated. As well, a vaporizer purchased from a online marihuana paraphernalia company and a water bong were also tested and analyzed. The results clearly showed that the pipes and water bong were all quite similar in cannabinoid profiles. However, the vaporizer consistently delivered less THC to the orifice. It is a hypothesis that based on the vacuum setting that was used, there is too much dead space in the vaporizer and hence at the vacuum setting used, there is not enough vacuum to sufficiently pull all the cannabinoids out of the orifice. This was partly confirmed by analyzing the inside of the vaporizer where significant amounts of cannabinoids were found bound to the glass enclosure. Furthermore, it is also possible that the glass enclosure requires conditioning first by repeated use so the wall of the container becomes saturated with THC. This may also lead to more THC then leaving the orifice after saturation has occurred.

In summary, experiments were carried out using both HPLC-UV and LC/MS/MS data on the combustion of marihuana cannabis plant and its associated cannabinoid profiles. It has been shown that there is a strong likelihood that the decarboxylation of THCA to THC during combustion is not a 100% conversion reaction. Furthermore, the data above suggest that the cannabinoids found in cannabis sativa plant are very heat

labile and hence trying to do quantification using gas chromatography systems may lead to inaccurate results. The current practice of injecting each analyte into the GC and then measuring its recovery is flawed as it does not take into account the varying rates and amount of thermal decomposition of each compound. Most interesting in this study was the conclusion that under a number of different conditions involving the combustion of THCA, the conversion to THC was always between 50-60%. Furthermore, using the same setup for combusting THC, the recovery was always greater then 90%.

6 FUTURE DIRECTIONS

One of the goals of this research paper was to validate the contents of combusted cannabis plant and to determine if there was a difference between combusted plant biochemical constituents versus uncombusted plant biochemical constituents. The research presented here clearly shows that further work in this area of study is required to further validate some of the conclusions mentioned above. Primarily, the next line of research should ask the question can the results found here be replicated in vivo? The human breathing model is certainly more complex then a simple smoking machine apparatus with respect to peak inspiratory volume, velocity and vital capacity. All of these factors could affect the results seen above. One of the primary goals of medical marihuana researchers is to determine if marihuana can be consumed via an alternative administration route besides inhalation. Recently, a number of pharmaceutical research companies have come up with products that are intended to do just that. In late 2005, GW Pharmaceuticals was approved by Health Canada to sell its sublingual spray that contains a 1:1 ratio of CBD and THC (also called cannabis medicinal extract or CME). As well, there is much research ongoing that is trying to reap the positive medical benefits of cannabinoids while removing the medically undesirable characteristics of marihuana.

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